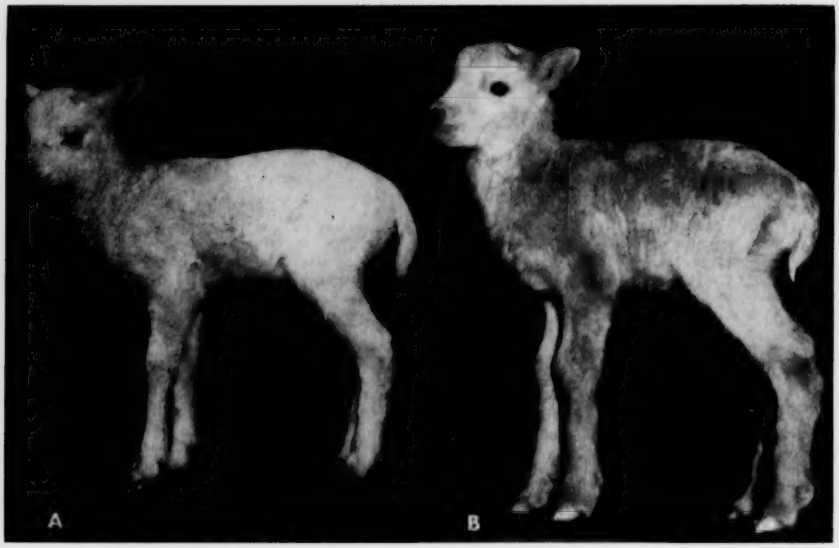
The Journal of HEREDITY



RECESSIVE BLACK IN SHEEP

(SEE PAGE 255)

Tripsacum-Corn Hybrids

Gene Studies in Tomato Hybrids

Black-Eyed White Mutation in Mice

Sex Chromosomes in the Maidenhair Tree

Selection for Larval Development Time in Drosophila

Pollen Cytology and Self-Incompatibility Systems in Plants

VOLUME 48 NOVEMBER-DECEMBER, 1957 NUMBER 6

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

WALTER E. HESTON, President — Vice-President, H. J. MULLER

Honorary Vice-President, W. E. CASTLE

SAMUEL L. EMSWELLER, Secretary — Treasurer, LAWRENCE G. BURK

ROBERT C. COOK, Editor — Managing Editor, BARBARA LAKE

EDITORIAL BOARD

J. T. BALDWIN, JR. — SHELDON C. REED — EARL L. GREEN — DELBERT T. MORGAN, JR.

JAY L. LUSH — HERBERT P. RILEY — JACK SCHULTZ

G. W. BEADLE — WALTON C. GREGORY — F. B. HUTT — G. LEDYARD STEBBINS

COUNCIL

RONALD BAMFORD, Dean of the Graduate School, University of Maryland, College Park, Md.

GEORGE M. DARROW, Horticulturist in Charge of Small-Fruit Breeding, Horticultural Field Station, Beltsville, Maryland.

SAMUEL L. EMSWELLER, Principal Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md.

BENTLEY H. GLASS, Professor of Biology, Johns Hopkins University, Baltimore, Maryland.

EARL L. GREEN, Director, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

JAY L. LUSH, Professor of Animal Breeding, Iowa State College, Ames, Iowa.

WALTER E. HESTON, Geneticist, National Cancer Institute, U. S. Public Health Service, Bethesda Md.

H. J. MULLER, Professor of Zoology, Indiana University, Bloomington, Indiana.

HUGH C. McPHEE, Assistant Director of Livestock Research, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

HAROLD H. PLOUGH, Department of Biology, Amherst College, Amherst, Massachusetts.

MARCUS M. RHOADES, Professor of Botany, University of Illinois, Urbana, Illinois.

LAURENCE H. SNYDER, Dean of the Graduate School, University of Oklahoma, Norman, Oklahoma.

ADVISORY COMMITTEE

R. A. BRINK, Professor of Genetics, University of Wisconsin, Madison.

LEONARD BUTLER, Professor of Genetics, University of Toronto, Canada.

TH. DOBZHANSKY, Professor of Zoology, Columbia University, New York City.

C. M. GOETHE, Sacramento, California.

JOHN W. GOWEN, Professor of Genetics, Iowa State College, Ames.

C. N. HERNDON, Dir., Out-Patient Dept., Bowman Gray School of Medicine, Winston-Salem, N. C.

HARRISON R. HUNT, Professor of Zoology, Michigan State College, East Lansing.

D. F. JONES, Plant Breeder, Connecticut Agricultural Experiment Station, New Haven.

M. A. JULL, Professor Emeritus of Poultry Husbandry, University of Maryland, College Park.

F. J. KALLMANN, M.D., Research Scientist, New York State Psychiatric Institute, New York City.

C. E. KEELER, Professor of Zoology, Georgia State College for Women, Milledgeville.

L. W. LAW, Senior Scientist (Geneticist), National Cancer Institute, Bethesda, Md.

PHILIP LEVINE, Director, Biological Division, Ortho Research Foundation, Raritan, N. J.

C. C. LITTLE, Director Emeritus, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

C. P. OLIVER, Professor of Zoology, University of Texas, Austin.

L. F. RANDOLPH, Professor of Botany, Cornell University, Ithaca, N. Y.

PAUL B. SAWIN, Research Associate, Jackson Memorial Laboratory, Bar Harbor, Maine.

KARL SAX, Professor of Botany, Harvard University, Arnold Arboretum, Jamaica Plain, Mass.

EDMUND W. SINNOTT, Professor Emeritus of Botany, Yale University, New Haven, Conn.

RAMSAY SPILLMAN, M.D., New York City.

B. L. WARWICK, Animal Husbandman and Geneticist, Texas Agri. Expt. Station, McGregor.

P. W. WHITING, Zoological Laboratory, University of Pennsylvania, Philadelphia.

G. W. WOOLLEY, Head, Division of Steroid Biology, Sloan-Kettering Institute, New York City.

CONWAY ZIRKLE, Professor of Botany, University of Pennsylvania, Philadelphia.

The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

Contents copyrighted 1957 by the American Genetic Association. Unauthorized reproduction of material from THE JOURNAL not permitted. The title "Journal of Heredity" is registered as a trade-mark in the U. S. Patent Office.

$7.00 a Year. $1.50 a copy. Prices of back numbers on application.

The Journal of Heredity

Vol. XLVIII NOVEMBER-DECEMBER, 1957 No. 6

CONTENTS

INHERITANCE OF RECESSIVE BLACK IN SHEEP
By Bruce L. Warwick, R. O. Berry and S. P. Davis 255

DIFFERENTIAL REACTION OF TWO VARIETIES OF SORGHUM TO COLCHICINE TREATMENT
By G. F. Atkinson, C. J. Franzke and J. G. Ross 259

SELECTION FOR LARVAL DEVELOPMENT TIME IN DROSOPHILA
By J. H. Sang and G. A. Clayton 265

POLLEN CYTOLOGY AND SELF-INCOMPATIBILITY SYSTEMS IN PLANTS
By James L. Brewbaker 271

A SELF-COMPATIBLE HYBRID FROM A CROSS BETWEEN TWO SELF-INCOMPARIBLE SPECIES IN TRIFOLIUM
By Kamla K. Pandey 278

PRENATAL MORTALITY IN MICE BEARING THE POLYDACTYL GENE COMPLEX
By Lawrence B. Hardy 282

GENE STUDIES IN TOMATO SPECIES HYBRIDS
By R. K. Soost and J. W. Lesley 285

THE SEX CHROMOSOMES OF THE MAIDENHAIR TREE
By Edward Gene Pollock 290

A NEW GENETIC FLOWER TYPE OF THE MUSCADINE GRAPE
By N. H. Loomis and C. F. Williams 294

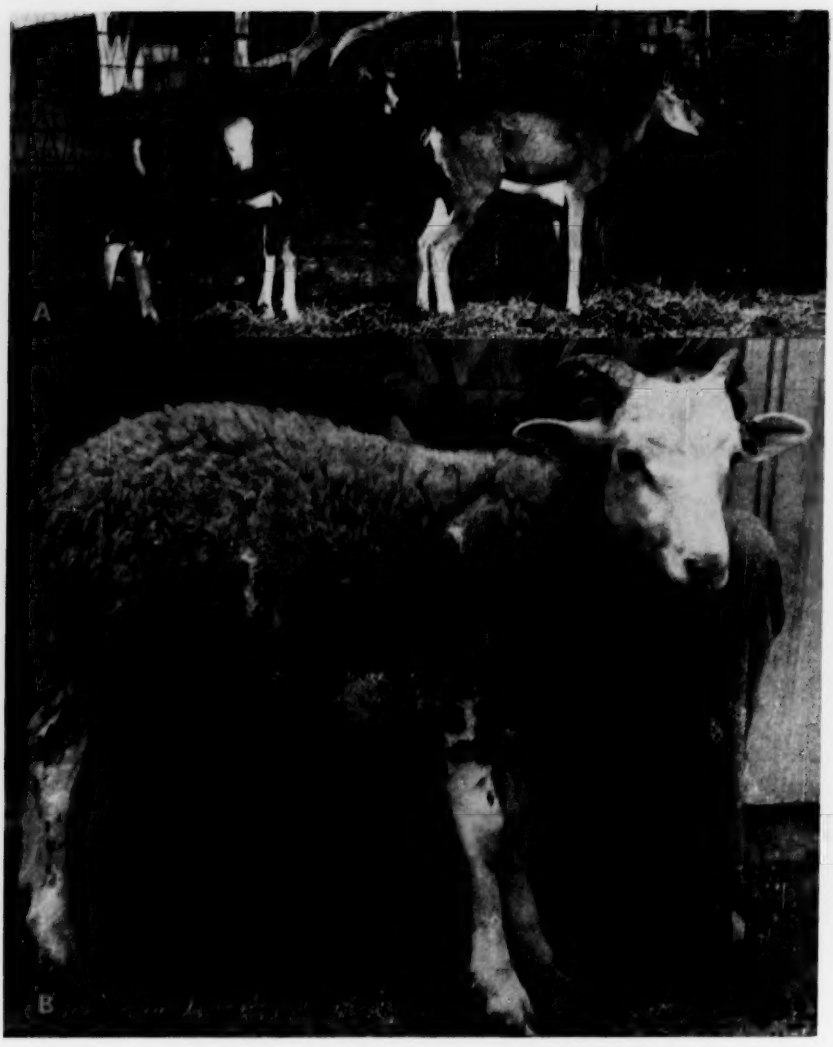
HYBRIDIZATION OF TRIPSACUM AND ZEA
By L. I. Farquharson 295

LINKAGE STUDIES IN A NEW BLACK-EYED WHITE MUTATION IN THE HOUSE MOUSE (NOT *W*)
By Philip C. Kreitner 300

Notes and Reviews

CORRIGENDUM 277

TITLE PAGE AND INDEX TO VOLUME 48 305



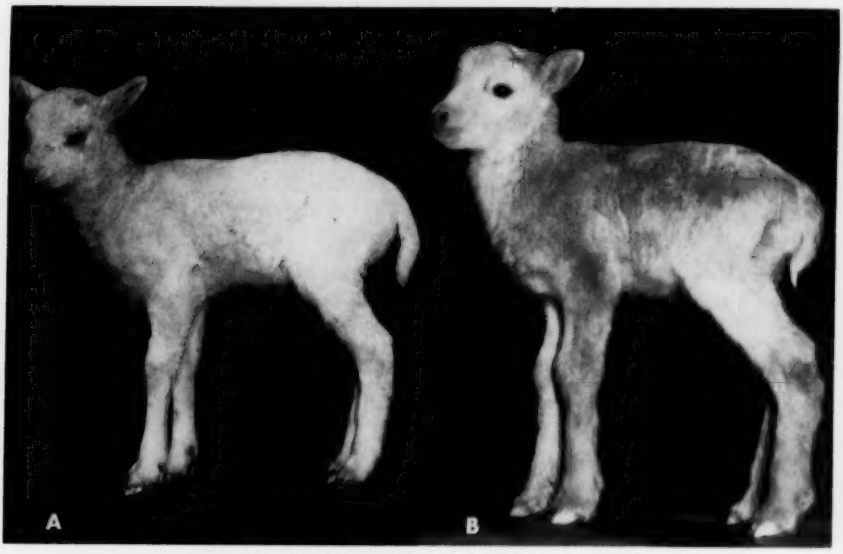
MOUFLON AND RAMBOUILLET × MOUFLON SHEEP

Figure 1

A—Mouflon sheep. These were raised in the San Diego Zoo and used for breeding experiments at the Texas Agricultural Experiment Station. They are basically recessive black with two pairs, $w_1w_1\,w_2w_2$. *B*—F_1 Rambouillet F × Mouflon M ram at about five months of age. Genotype $W_1w_1\,W_2w_2$.

INHERITANCE OF RECESSIVE BLACK IN SHEEP

BRUCE L. WARWICK, R. O. BERRY AND S. P. DAVIS*
Texas Agricultural Experiment Station



TYPICAL RAMBOUILLET-MOUFLON LAMBS

Figure 2

A—A typical F_1 Rambouillet F × Mouflon M new-born lamb. This white lamb has the genotype $W_1 w_1 W_2 w_2$. *B*—shows lambs #603-S10 which is ¾ Mouflon and ¼ Rambouillet. The sire is the ram shown in Figures 1*B* and 2*A* and the mother is the ewe in Figure 1*A*. The genotype of this lamb is $W_1 w_1 w_2 w_2$.

BLACK lambs are commonly seen in white flocks, even where none are retained for breeding. These have been interpreted by Davenport[2] and others as recessive black. Recessive black is seen in most if not all breeds of sheep common in this country. Most of the sheep raised in the United States are of fine-wool breeding, i.e. Rambouillet or Merino and the occasional black lamb is considered to be a recessive segregate. Several technical reports on the inheritance of this color in sheep have included one or more "exceptions" to the expected black lambs when both parents were black[1, 2, 3, 5, 6]. These have been explained as possibly due to errors in records or to management accidents. The reversal of dominance has also been suggested. In connection with another experiment we obtained results which suggested the existence of more than one pair of recessive genes, any one of which when homozygous would result in black offspring. If this hypothesis is valid, the above white exceptions could result from the mating of recessive black due to one pair of genes ($w_1 w_1 W_2 W_2$) to recessive black due to another pair of genes ($W_1 W_1 w_2 w_2$). This was re-

*Animal Husbandman and Geneticist, McGregor, Texas; Professor of Animal Husbandry, College Station, Texas; and Wool Technologist, College Station, Texas, respectively.

WHITE AND DILUTE BLACK

Figure 3

A—White lamb resulting from mating recessive black ewe × ram 603-S10 (Figure 2*B*). The genotype of the ewe is $w_1 w_1 W_2 w_2$. The genotype of the sire is $W_1 w_1 w_2 w_2$ and the genotype of the lamb is $W_1 w_1 W_2 w_2$. *B*—The same ram as shown in Figure 2*B* at the age of one year, nine months. This ram was "brownish" in color which was a dilute black. The genotype is $W_1 w_1 w_2 w_2$.

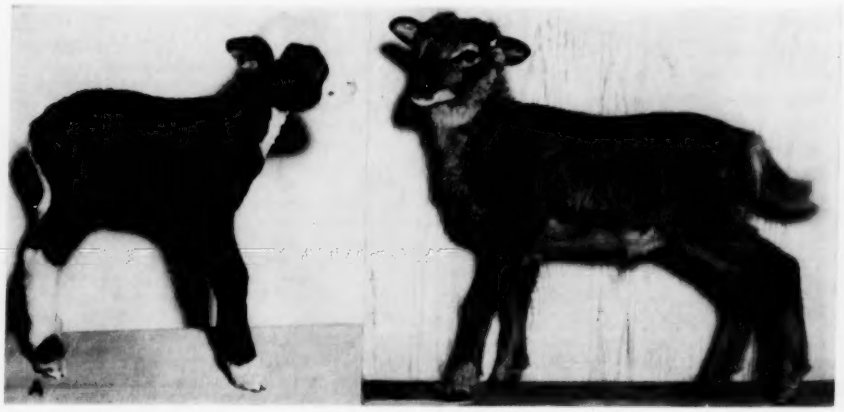
ported in abstract form, Warwick *et al.*[9] but the full account of the evidence has not yet been published.

Source of Data and Analysis

A registered polled Rambouillet ram, TLD 590, purchased by the Texas Agricultural Experiment Station for experiment in a study of the inheritance of hornlessness, proved to be homozygous for the dominant gene of hornlessness *(HH)*[10]. However, he was also found to be a carrier of recessive black as shown by segregation of black when he was bred to his daughters, (Table I). Several of his sons were also carriers of recessive black. Because this was the first polled Rambouillet proven to be homozygous for hornlessness, he was also used in the flock of a cooperator, who recognized that half of the sons of TLD 590 would be carriers of recessive black. He tested several sons of TLD 590 by breeding to black ewes which he purchased from several large fine-wool flocks where they had been "cropping-out." It was assumed that they were black due to the same genes as carried by TLD 590 ($w_1 w_1$). One of the rams sired eight white lambs from seven black ewes the first season. While this was considered a good test it was decided to repeat the test the next season, and also to breed this ram to white registered Rambouillet ewes. Ten white lambs from seven black ewes the second season

TABLE I. Progeny of ram TLD 590 and two sons all heterozygous for recessive black (W_1 w_1 W_2 W_2) mated to white daughters of two of these

Rams	Ewes	Lambs		Assumed	P
		White	Black	Ratio	
TLD 590	Daughters of 590	17	2	7:1	.2760
TS 1464	Daughters of 590	8	1	7:1	.3866
SSB 4239	Daughters of 590	8	0	7:1	.3436
SSB 4239	Daughters of 1464	2	2	7:1	.0718
Totals		35	5	7:1	.1875



BLACK AGOUTI

Figure 4

A—Black segregate resulting from mating F_1 ram $(W'_1 w_1 W'_2 w_2)$ to a Rambouillet ewe which was a carrier of recessive black. The genotype of this lamb is probably $(w_1 w_1 W'_2 w_2)$. *B*—Example of a black agouti segregate. The dam of this lamb was a white ewe sired by a son of TLD 590, and the sire was 603-S10. Agouti is a pattern gene whose effect is only visible in animals segregating for color (black in this case).

brought the tally for the two years to 18 white lambs from 12 black ewes. (Table II). Clearly, this would be considered "conclusive" with the probability of such occurrence less than 1 in 10,000[7]. However, included in the group of white registered Rambouillet ewes to which he was bred were 16 ewes belonging to the Experiment Station, 11 of which were sired by either TLD 590 or by another son of TLD 590, known to be a carrier of recessive black. These 11 ewes produced 10 white lambs and two black lambs. (Table I). This was evidence which suggested the existence of at least two independent pairs of recessive genes (w_1 w_1 and w_2 w_2), either pair when homozygous resulting in black lambs.

The above black tester ewes were added to a group of black ewes from the same and similar sources which we were using to test rams for recessive black. We had a black segregate ram 465-S10 which was assumed to carry the same genes for recessive black as were carried by TLD 590. However, there were three intervening generations through his sire between him and TLD 590. His ma-

TABLE II. Test matings of ram SSB 4239 $(W_1 w_1 W_2 W_2)$ to recessive black ewes (assumed genotype $w_1 w_1 W_2 W_2$)

Ram	Ewes	Lambs		Assumed	
		White	Black	Ratio	P
SSB 4239	Tester ewes	18	0	1:1	.0000

TABLE III. Progeny of recessive black ewes × recessive black rams

Ram	Ewes	Lambs		Assumed	
		White	Black	Ratio	P
465-S10	Tester ewes	0	22	All black	1.0000
603-S10	Tester ewes	4	3	All black	.0000

ternal granddam was one of the black tester ewes. This ram was bred to a number of the black tester ewes to determine whether some of them differed enough genotypically from this ram to produce white lambs. In every case the offspring was black. Twenty-two black lambs were produced, (Table III). Evidently, the same genes were present in the ewes, as in this particular sire.

Discussion

It was shown by Roberts[4] that the color of the wild Mouflon *(Ovis musimon)* is basically recessive black, modified by pattern genes. This was based on the study of the offspring of a Mouflon ram and domestic ewes on Lambay Island off the eastern coast of Dublin after the war time hunting and killing of all the Mouflon ewes on the island. The Texas Agricultural Experiment Station has bred Mouflon rams to white Rambouillet ewes. In every case the offspring was white, although in a few cases there was a small amount of black spotting. Also, infantile red or brown spotting which disappeared as the lamb grew older was fairly common. In the backcross to the Rambouillet, black segregated when white carrier ewes for recessive black were involved. This was all in agreement with the interpretation of a single pair of recessive genes for black *($w_1 w_1$)*.

With the evidence presented above suggesting, but not proving, that there were two or more independent pairs of recessive genes for black, it was logical to raise the question as to whether the Mouflon, which was one of the principal progenitor species[8], might not carry the two recessive pairs *($w_1 w_1 w_2 w_2$)* rather than one of the genes being a more recent mutation. Among the experimental crosses of Mouflon and Rambouillets, we had a ram which was the result of backcrossing a pure Mouflon ewe *($w_1 w_1 w_2 w_2$)* to an F_1 Rambouillet $\times$ Mouflon ram *($W_1 w_1 W_2 w_2$)*. This ¾ Mouflon, ¼ Rambouillet ram was of a light "brownish" color, which was a dilute black. Theoretically, if the two-pair hypothesis was followed, this ram could be homozygous for both pairs, *($w_1 w_1 w_2 w_2$)* or homozygous for one of the pairs and heterozygous for the other pair, *($W_1 w_1 w_2 w_2$* or *$w_1 w_1 W_2 w_2$)*. It was decided to put this to test by breeding the ram to as many black ewes of different origin as possible. The birth of even one white lamb from such a mating would definitely prove the existence of two independent pairs of recessive genes. Four such white offspring were produced, (Table III). The accompanying illustrations and tables illustrate the material and supply the data on which the conclusions have been based.

Summary and Conclusions

Recessive black commonly found segregating in flocks of white sheep has long been interpreted as due to a single pair of genes, even though more than one investigator reported "exceptions." A Rambouillet ram tested by siring 18 white lambs from black tester ewes was considered to be free from the recessive black gene until further breeding evidence revealed his heterozygosity for a pair of recessive genes. Matings to half sisters and to daughters of a half brother produced two black segregates, which suggested the existence of two pairs of recessive genes for black, either of which when homozygous would result in black *($w_1 w_1$* and *$w_2 w_2$)*. This was tested by mating recessive black rams to recessive black ewes of different origins. One of the black (dilute) rams, ¾ Mouflon and ¼ Rambouillet *($W_1 w_1 w_2 w_2$)* when mated to black ewes *($w_1 w_2 W_2 -$)* sired three black and four white lambs proving the existence of two pairs of recessive genes in domestic sheep. It is postulated further that these two pairs of recessive genes for black *($w_1 w_1 w_2 w_2$)* were present in the primitive Mouflon species of sheep which was the principal progenitor of both the Mouflon *(Ovis musimon)* and the fine-wool breeds (Merino and Rambouillet).

Literature Cited

1. Castle, W. E. Genetics of the multinippled sheep. *Jour. Hered.* 15: 75-85. 1924.

2. Davenport, C. B. The origin of black sheep in the flock. *Science* N. S. 22: 674-675. 1905.

3. Rae, A. L. The genetics of sheep. *Advances in Genetics* 8: 193-198. 1956.

4. Roberts, J. A. Fraser. Colour inheritance in sheep. VI. The genetic constitution of the wild mouflon. *Jour. Genet.* 25: 1-16. 1932.

5. ———— and R. G. White. Colour inheritance in sheep. IV. White, recessive black, recessive brown, badger-face pattern and reversed badger-face pattern. *Jour. Genet.* 22: 181-190. 1930.

6. Warwick, B. L. Breeding experiments with sheep and swine. *Ohio Agr. Exp. Station Bul.* 480. 1931.

7. ————. Probability tables for Mendelian ratios with small numbers. *Texas Agr. Exp. Station. Bul.* 463. 1932.

8. ———— and R. O. Berry. The origin and early history of sheep. *Sheep and Goat Raiser* 22: 33. 1941.

9. ————, R. O. Berry and S. P. Davis. Hybridization of wild Mouflon $\times$ domestic sheep. *Jour. Animal Sci.* 5: 412-413. 1946.

10. ————, J. M. Jones, W. H. Dameron and P. B. Dunkle. Polled Rambouillet Breeding. *Proc. Soc. An. Prod.* pp. 287-289. 1933.

DIFFERENTIAL REACTION OF TWO VARIETIES OF SORGHUM TO COLCHICINE TREATMENT

G. F. Atkinson, C. J. Franzke and J. G. Ross*

THE induction of true-breeding diploid mutants in sorghum by colchicine treatment has been reported by Franzke and Ross[3] and by Ross *et al.*[8] Meiotic studies of the mutant plants, untreated plants and their F_1 hybrids by Harpstead *et al.*[5] gave no evidence of any rearrangements of the chromosomes or of any cytological irregularities. Genetic studies of F_2 populations of crosses between mutant and untreated plants[2] indicated that mutations had occurred at a number of loci. True-breeding mutant lines induced by colchicine treatment were found to mutate to new true-breeding lines when treated again[4].

It has been suggested that mutant plants might result from point mutations made homozygous by a somatic reduction of the chromosomes (of the nature of a reductional grouping as described by Huskins[6]) followed by a doubling of the chromosome number[4]. A cell so formed might organize a new growing point after the original one had been inactivated during c-tumor formation. The occurrence of homozygous parental genes in chimeral sectors of a treated F_1 flax plant originally heterozygous (Dirks *et al.*[1]) indicates that reduction with subsequent doubling is not necessarily associated with the mutagenic effect.

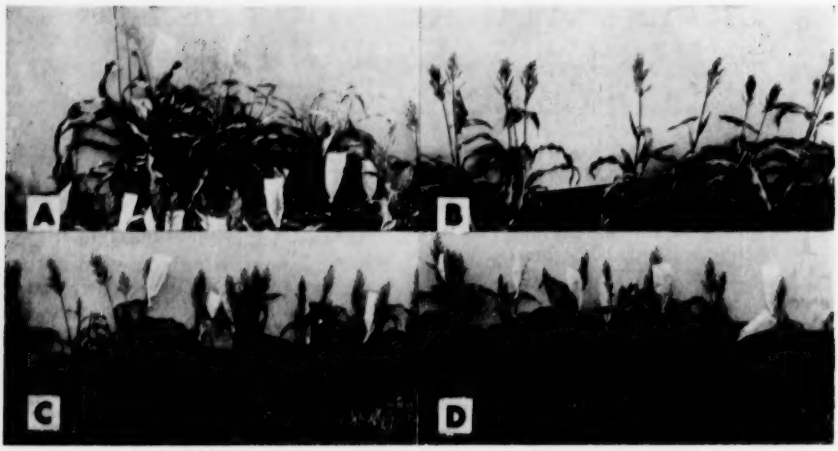
During the course of routine work with colchicine-treated sorghum it was noted that some varieties, after colchicine treatment, gave tetraploids with few or no gene mutations. The present studies were undertaken to demonstrate differences between two varieties of sorghum in their reaction to colchicine.

Materials and Methods

The two varieties of sorghum, Experimental 3 and Norghum, used in this study are true-breeding, dwarf, grain types. The origin of Experimental 3 has been described previously[3]. Norghum is a selection from the cross of a selection from (Dwarf Feterita × Dwarf Freed) with Yellow Kaffir. The stocks of each variety used had been self pollinated for more than 10 generations.

Sixty seedlings of each variety were treated with colchicine by the method described by Franzke and Ross[3]. Surviving seedlings were transplanted to the field together with untreated plants of each variety for comparison. Heads were collected from untreated and treated plants for chromosome counts. Notes were taken on individual plants for height, number of tillers, presence or absence of awns and aberrant plant characters. Similar measurements were made on selfed progenies of these plants grown in the field the following year. Chromosome counts were made at meiosis in the pollen-mother-cells. Untreated and colchicine-treated root tips were examined to determine the frequencies and types of reductional groupings of the two varieties. Roots were cut off germinating seedlings and equal numbers placed in 0.5 percent aqueous solution of colchicine and in tap water for one and two hours. After treatment the roots were fixed in hot (60° C.) 3:1 alcohol-acetic acid for 15 minutes and stained with Feulgen stain. The root tips were then squashed in aceto-carmine and examined.

*Agronomy Department, South Dakota State College, Journal Paper Number 373, South Dakota Agricultural Experiment Station, Brookings, South Dakota. This investigation was supported by research grant P. H. S. C2086 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.



DIFFERENTIAL MUTAGENIC EFFECT OF COLCHICINE

Figure 5

A—Shows individual plants of Experimental 3 after treatment with colchicine, illustrating mutations with unchanged chromosome number that take place as a result of treatment. *B*—Shows untreated plants of the same variety. *C* and *D*—Show treated and untreated plants of the variety Norghum illustrating the lesser mutagenic effect of colchicine on this particular genotype.

Observations and Discussion

Of the 60 seedlings of each variety treated in the spring of 1955, 52 Experimental 3 and 54 Norghum survived and were transplanted to the field, also 35 untreated plants of each variety were planted for comparison. Forty-three treated Experimental 3 and 54 treated Norghum plants survived to maturity and were studied. Photographs of untreated Experimental 3, treated Experimental 3, untreated Norghum and treated Norghum are shown in Figure 5. The variability of the treated Experimental 3 plants as compared to the other groups is clearly evident.

Thirty treated Experimental 3 plants examined cytologically were all found to be diploid. Three out of 30 treated Norghum plants proved to be tetraploid. Ten untreated Experimental 3 and 15 untreated Norghum plants were examined; all were found to be diploid. No irregularities at meiosis were noted in any of the figures examined.

Data on presence or absence of awns, height and number of tillers of untreated and treated Experimental 3 and Norghum are summarized in Table I. Sieglinger, Swanson and Martin[9] have found absence of awns to be a simple dominant to presence of awns. Awns were

TABLE I. Number of awned plants, height and number of tillers of untreated and colchicine-treated plants of Experimental 3 and Norghum

	No. of plants	No. of awned plants	Height in inches			Number of tillers		
			Mean	Range	Variance	Mean	Range	Variance
Experimental 3								
Untreated	26	0	24.6	20-38	24.63	2.5	1-4	1.06
Treated	43	14	37.5	21-57	81.30	7.5	0-25	63.83
t			1.60			1.51		
F					3.30**			60.22**
Norghum								
Untreated	34	34	32.5	26-40	13.65	5.1	3-9	1.88
Treated	54	54	29.1	22-36	13.26	3.8	1-8	3.59
t			4.28**			3.21**		
F					0.97			1.91

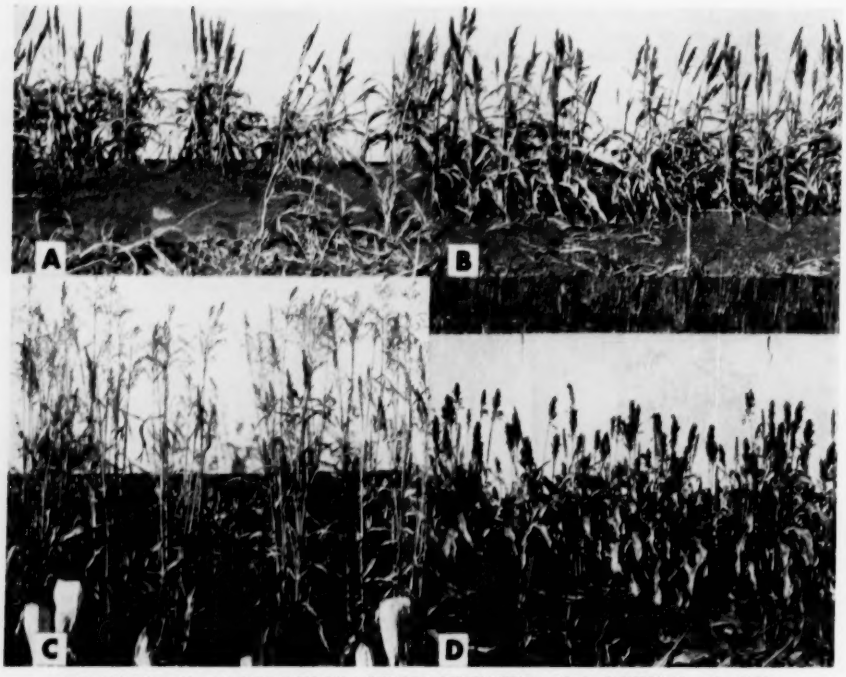
**Significant at the 1 percent level.

observed on 14 out of 43 treated plants, while none were found on the 26 untreated plants. The lack of a significant difference (as indicated by the t test) between untreated and treated Experimental 3 for height and number of tillers, despite the great apparent differences, is due to the extremely high variance of the treated plants. The significant differences between the means of untreated and treated Norghum is undoubtedly due to the adverse effects of colchicine on the treated plants in the seedling stage; the untreated plants were taller and had more tillers than the treated plants. The F test shows a highly significant difference between the variances of untreated and treated Experimental 3 but no significant difference in variability between the two groups of Norghum plants. This significantly greater variance of the phenotype of Experimental 3 for presence or absence of awns (obviously significant) height, and number of tillers, would seem to indicate that as a result of treatment, mutations have taken place to a much greater degree in this variety than in Norghum.

Comparisons of the genotypes were made by observing the progenies of these treated and untreated plants; data from representative samples are shown in Table II. Distinct differences were found between and within progenies of treated plants of Experimental 3. In many cases a marked difference in height and panicle type is found between the progeny of a treated plant and that of an untreated plant as illustrated in Figure 6. Among 43 progenies of treated plants of Experimental 3, of which those shown in Table II are a sample, 24 were different from those of the untreated Experimental 3; 18 of these bred true for all changed characteristics. The highly significant parent-progeny correlation coefficient for height of the treated Experimental 3 (Table III) indicates that the height differences of the treated plants were due to different genotypes. It may be concluded that these differences were induced by colchicine treatment since the untreated plants do not differ in height, nor is the parent-progeny correlation of the untreated plants significant. On the other hand, the apparent differences between and within progenies of the treated Norghum plants were much less evident as shown in Table II. One

TABLE II. Comparison of representative samples of treated and untreated Experimental 3 and Norghum plants and their selfed progenies

Plant no.	Haploid Parent chromosome no.	Plants in progeny	Height in inches		Awns			Observations on progeny
			Parent	Progeny	Parent	Progeny Present	Progeny Absent	
Experimental 3								
Treated								
1		11	42	41.6±4.6	absent	2	9	Segregating for mutations
2	10	24	30	29.7±3.3	absent	0	24	No detectable mutation
3		44	26	29.3±4.2	absent	0	44	No detectable mutation
4	10	16	25	32.1±8.3	absent	1	15	Segregating for mutations
5		27	28	27.6±6.2	absent	1	26	Segregating hairy glumes
6	10	26	35	34.3±9.2	present	26	0	True-breeding for mutations
7	10	26	46	46.1±6.3	present	26	0	True-breeding for mutations
8	10	12	47	42.8±1.6	present	12	0	True-breeding for mutations
Untreated								
1		25	36	28.5±3.8	absent	0	25	No detectable mutation
2	10	18	37	24.0±2.7	absent	0	18	No detectable mutation
3	10	16	36	29.8±3.2	absent	0	16	No detectable mutation
4	10	22	33	26.5±2.8	absent	0	22	No detectable mutation
5	10	22	34	28.0±4.4	absent	0	22	No detectable mutation
Norghum								
Treated								
1	10	17	34	39.9±2.0	present	17	0	No detectable mutation
2	10	15	24	40.6±4.6	present	15	0	No detectable mutation
3		23	33	38.3±4.2	present	23	0	No detectable mutation
4		29	32	41.4±5.3	present	29	0	Segregating for head type
5	10	16	32	37.4±5.0	present	16	0	No detectable mutation
6	10	12	27	38.1±2.5	present	12	0	No detectable mutation
7		19	27	40.2±4.7	present	19	0	No detectable mutation
8	10	14	28	42.4±8.4	present	14	0	One tall plant
Untreated								
1	10	25	30	43.7±8.2	present	25	0	No detectable mutation
2	10	14	26	39.1±1.2	present	14	0	No detectable mutation
3	10	18	27	41.2±2.9	present	18	0	No detectable mutation
4	10	21	28	41.4±3.3	present	21	0	No detectable mutation
5	10	18	30	42.0±3.3	present	18	0	No detectable mutation



COMPARISON OF PROGENIES FROM MUTATED AND NORMAL PLANTS

Figure 6

Progenies *A* and *C*, each from a treated plant, and *B* and *D* each from an untreated plant of the variety Norghum above, and Experimental 3 below, illustrate a change in genotype without change in chromosome number of certain treated plants. Though Norghum responded to colchicine treatment by forming tetraploids in approximately 10 percent of the cases, there were also four diploid mutants among the 45 treated plants. In Experimental 3 no polyploid plants have ever been found after treatment; in this instance 23 mutants out of 43 treated plants were observed.

progeny with small differences in head type, another with 12 white-seeded plants out of 25, and two progenies of uniform shortness similar to the progeny shown in Figure 6 were observed. Only four diploid progenies out of 45 were observed to have changes that seem to have arisen from mutation. However, other treated progenies contained individual tall plants which may have resulted from poor selfing technique or, on the other hand, may be mutations since similar types were not observed in the progenies of the untreated plants selfed in the same fashion. The parent-progeny correlation coefficients for the height of this variety were not significant (Table III) indicating that, in general, height differences be-

TABLE III. Correlation coefficients of height of treated and untreated parent plants and their selfed progenies in Experimental 3 and Norghum

	N	Correlation coefficient
Experimental 3		
Treated	26	.819**
Untreated	7	.412
Norghum		
Treated	45	—.237
Untreated	10	.158

**Significant at the 1 percent level.

tween the treated plants were not of genetic origin.

As mentioned above, one of the differences in reaction of these two varieties to colchicine treatment appears to be readiness to form tetraploids. In the case of Norghum, 10 percent of the treated plants examined were tetraploid, while none were found in an equal number of treated plants in Experimental 3. Tetraploid plants have not been found among the many treated plants of this variety previously examined. The mutagenic effect of colchicine is more evident in Experimental 3 than in Norghum, but the difference appears to be only in degree since mutations are found in both varieties. No difference in degree of induced homozygosity could be ascertained since approximately the same proportion of the mutants were true-breeding in each variety. In Experimental 3, 18 of the 24 mutant progenies were uniform, while in Norghum, two of the four aberrant progenies were uniformly shorter than the untreated.

This genotypic difference in reaction to colchicine may account, partially at least, for the paucity of references in the colchicine literature to aberrant forms other than polyploidy arising from treatment. This difference would also lead to varying results of different investigators testing the effects of colchicine on plant materials. Besides the necessity for an appropriate genotype to form a favorable internal environment, it appears from work now in progress that a favorable external environment after application of colchicine must also be provided if the investigator is to be successful in obtaining true-breeding mutant types even from Experimental 3. Further investigations are now under way to ascertain these conditions.

A clue that might lead to an understanding of the basis for the difference in response to colchicine treatment was sought by observing the effect of colchicine on the chromosomes of the root tips from each of these varieties. Reductional groupings, similar to those noted by Huskins[6] were found in the treated roots of both. Table IV shows the number of cells with reductional groupings in relation to the total number of divisions. The slight difference in frequency of reductional groupings between Experimental 3 and Norghum does not appear to be great enough to be significant. In both varieties, great variations in the number of reductional groupings were found between root tips. In both treated Experimental 3 and Norghum, five of the reductional groupings occurred in one root.

In most of the cells with reductional groupings, there were two groups of chromosomes, but in a number of cases, there were more than two distinct groups; these are included in the class "multiple groups" in Table V. Where only two groups were present, there was considerable variation in the exactness of the reduction. Reductional groupings with two groups were therefore subdivided according to the number of chromosomes in each group.

In both the treated Experimental 3 and the treated Norghum there appears to be a concentration of reductional groupings in the classes having two groups of equal or approximately equal numbers of chromosomes (10:10, 9:11 and 8:12).

In the majority of cases it was found impossible to distinguish any of the homologous

TABLE IV. Frequency of reductional groupings in untreated and colchicine-treated root tips of Experimental 3 and Norghum

	No. of roots	Total no. of divisions	Number of reductional groupings	Percent reductional groupings
Experimental 3				
Untreated	24	128	2	1.6
Treated	29	273	25	8.4
Norghum				
Untreated	21	78	0	0
Treated	23	189	15	7.9

TABLE V. Distribution of types of reductional groupings in untreated and colchicine-treated root tips of Experimental 3 and Norghum

	Multiple groups	Two groups							Total
		4:16	4:15	6:14	7:13	8:12	9:11	10:10	
Experimental 3									
Untreated				1	1				2
Treated	4	1		1	2	6	4	7	25
Norghum									
Untreated									0
Treated	2	1	1	1	2	3	2	3	15

chromosomes, even the long pair called the A-pair by Huskins and Smith[7]. In one reductional division of the 10:10 class in a treated Norghum root tip, the members of the A-pair could both be distinguished and were both in the same group. Since, except for this one case, homologous chromosomes could not be identified, no estimate of the degree of homologous segregation in the reductional groupings could be made.

The basic cause for the observed differences in reaction to colchicine of these two varieties of sorghum does not appear to be in the initial behavior of the chromosomes after colchicine treatment. It may perhaps be found in varietal differences within the colchicine-induced tumor where tissues are subject to the prolonged effect of colchicine present in the lanolin adhering to the coleoptile. An account of a histological study of c-tumors induced in seedlings of these sorghum varieties will be made elsewhere.

Summary

Colchicine treatment of the coleoptiles, immediately after germination, of 60 seedlings of each of two varieties of grain sorghum, Experimental 3 and Norghum, resulted in the mutation of 24 out of 43 surviving Experimental 3 plants and of four out of 54 surviving Norghum plants. Cytological examination at meiosis of 30 treated plants of each variety indicated only diploid plants in Experimental 3 but three tetraploid plants in Norghum. Previous examinations of meiosis of treated plants of Experimental 3 have likewise shown no instance of polyploidy. In response to colchicine treatment, these varieties differ in mutation rate and in propensity to polyploidy. Such differences explain, partially at least, inconsistencies in results of treating different genotypes. Analysis of response of the chromosomes in the root tips of these two varieties to an aqueous solution of 0.5 percent colchicine showed no difference in the frequency of reductional groupings.

Literature Cited

1. Dirks, V. A., J. G. Ross and D. D. Harpstead. Colchicine-induced true-breeding chimeral sectors in flax. *Jour. Hered.* 47: 229-233. 1956.

2. Foster, A. E., J. G. Ross and C. J. Franzke. Genetic analysis of F_2 populations from crosses involving colchicine-induced mutants in sorghum. (Abstract) *Proc. S. D. Acad. Sci.* 34: 21-22. 1955.

3. Franzke, C. J. and J. G. Ross. Colchicine-induced variants in sorghum. *Jour. Hered.* 43: 107-115. 1952.

4. ————. A lineal series of new mutants induced by colchicine treatment. *Jour. Hered.* 48: 47-50. 1957.

5. Harpstead, D. D., J. G. Ross and C. J. Franzke. The nature of chromatin changes of colchicine-induced variants in sorghum. *Jour. Hered.* 45: 255-258. 1954.

6. Huskins, C. Leonard. Segregation and reduction in somatic tissue. *Jour. Hered.* 39: 311-325. 1948.

7. Huskins, C. Leonard and S. G. Smith. A cytological study of the genus *Sorghum* Pers. I. The somatic chromosomes. *Genetics* 25: 241-249. 1932.

8. Ross, J. G., C. J. Franzke and L. A. Schuh. Studies on colchicine-induced variants in sorghum. *Agron. Jour.* 46:10-15. 1954.

9. Sieglinger, J. B., A. F. Swanson and J. H. Martin. Inheritance of awn development in sorghums. *Jour. Agr. Res.* 49: 663-668. 1934.

SELECTION FOR LARVAL DEVELOPMENT TIME IN DROSOPHILA

J. H. Sang and G. A. Clayton*

ONE reason that *Drosophila* studies have contributed less than might have been expected to quantitative genetics lies in the difficulty of finding "characters" which are amenable to experimentation. Wing and thorax length[13] and bristle numbers[6, 10, 2] have been studied extensively, but the more complex attributes such as fecundity and fertility, which are intimately related to fitness, have been somewhat ignored due to the technical difficulties associated with their measurement. Yet as Bell, *et al*[1] and Tantawy and Reeve[17] have shown for egg number in *Drosophila*, it is this kind of trait which most resembles the many important production characteristics of domestic animals (e.g. egg production in fowls, litter size in pigs, etc.) which are notably intractable to the usual selection procedures. As normally assessed, these traits related to fitness show a low heritability and some degree of hybrid vigor[1] as also do the production characters cited. There is therefore a real need for more studies of "characters" which behave in this way. This report records some preliminary experiments with a character of *Drosophila* which was expected to have these qualities but which can be readily handled experimentally; namely, duration of larval life.

Materials and Methods

A preliminary selection experiment was run using a vigorous mass mated strain recently introduced into the laboratory (Wild Edinburgh). The main tests were made with a line which had previously been selected for large wing size and then made isogenic (Edinburgh Large) and an inbred Ore K line reputedly sib mated for 756 generations. Selection was started from the F_2 of a cross between these two lines.

Eggs were collected as described by Robertson and Sang[12] and the larvae picked off as they hatched. Fifty larvae were placed in 3×1 inch vials each containing 5 ml. of Pearl's S101 semi-synthetic medium, as modified by Robertson and Sang[12], which had been inoculated with 70 mgms. of dried Brewer's Yeast just prior to the introduction of the larvae. The cultures were prepared, kept and scored in a constant temperature room at 75° F ± 2° F: even so, the great sensitivity of the larval period to temperature[9] made it essential that only contemporary cultures be compared, as is the case in the results which follow. The end of the larval period was taken as the time of eversion of the spiracles shortly after the larva becomes immobile. Scoring was done as frequently as possible, with never more than a five hour gap between counts. Familiarity with the material made possible closer timing than this since the gradual darkening of the pupa case could be used to age the animals to the nearest hour.

As all larvae had to be set up within a short period (which was taken as not exceeding two hours) the number of females used for each generation had to be kept high. It was found that 50 pairs of parents, if carefully nourished and used at peak lay (4 to 7 days old), would yield sufficient eggs to set up 500 larvae the next day. Selection was consequently arranged so that the first (or last) hundred pupae formed out of 500 larvae set up were used as parents for the next generation. Adjustments were made, of course, for any differences between the

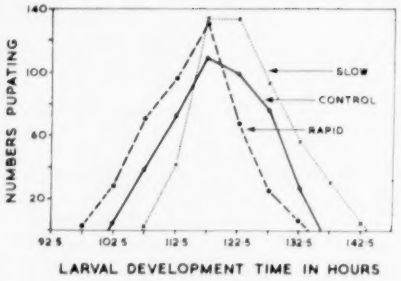
*Agricultural Research Council, Poultry Research Centre, Edinburgh 9; and Institute of Animal Genetics, Edinburgh 9, respectively.

times at which the individual vials were set up. The use of 100 parents eliminated any inbreeding effects. It must also be noted that the food environment supplied by the cultures was somewhat sub-optimal[14] so mortality changes or differences may introduce further variables into the experiments: these are considered below.

Observations

Since no genetic data on duration of larval life were available, a preliminary selection experiment was set up to assess the suitability of the techniques and the biometrical properties of the character. Three sets of 10 vials were prepared and innoculated with Wild Edinburgh larvae. From the first set the 100 earliest pupae were selected to give a "rapid line"; from the second, the last 100 pupae were selected to give a "slow line", and the third set was kept unselected as the control. The three populations were measured at the third generation of selection with the results shown in Figure 7, which indicates that duration of larval life has a unimodal distribution. The discontinuities and the sex differences found when the entire pre-adult period is measured[8] are not observed for the larval stage alone, and must therefore arise during the pupal-instar.

The data (Table I) show, too, that the character has responded symmetrically to selection, and that variances are not affected whether calculated on normal or logarithmic scales. The ratio of the



LARVAL DEVELOPMENT TIME

Figure 7

The distributions of larval development times after two generations of selection for rapid and for slow growth, are compared with an unselected control population.

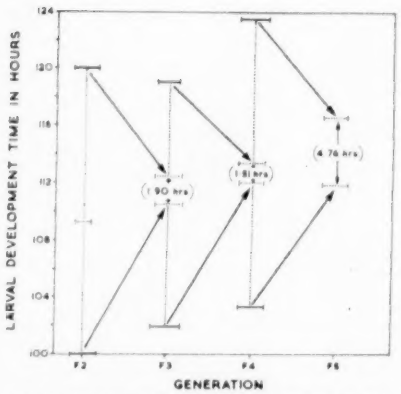
sum of selection differentials (computed on the basis of an assumed normal distribution and a selection intensity of 23 percent to allow for non-specific larval mortality) to the actual response gives an estimate of the heritability of the character of 21.0 percent, which was higher than anticipated and falls within the intermediate rather than the low heritability range.

A more extensive selection program was initiated from the F_2 of a cross between the Edinburgh Large isogenic, and the Ore K inbred, lines: the F_2 being chosen since much selection for characters of economic importance starts from a cross of improved lines or varieties. The techniques and intensity of selection were described above, except

TABLE I. Larval development times at the 3rd generation of selection. (Wild Edinburgh stock)*

	Control	Rapid	Slow
Larval development time (hours)			
mean	119.5	115.3	122.9
variance (s^2)	50.13	48.90	46.20
number	426	426	492
Larval development time (log. days)			
mean	0.696	0.680	0.710
variance (s^2)	0.00063	0.00066	0.00059

*There are reasons[15] for measuring larval development times on a logarithmic scale since this tends to normalize distributions, particularly when growth is greatly prolonged. In this instance the scalar change has no important effect. Since not all larvae survive, the selection intensity of 20 percent originally scheduled works out at 23 percent in practice. By comparing the variances of a wild stock and an F_1, F. W. Robertson (unpublished) estimates the heritability of this character at around 50 percent. The same estimate is obtained from the data given here and in Table II, if development is measured in hours. When measured in log. days the heritability is about 27 percent by this assessment.



SELECTION FOR RAPID AND SLOW DEVELOPMENT

Figure 8

Progress of selection for rapid and slow larval development starting from an F_2 between Edinburgh Large and Ore K homozygous lines. The means of each generation are shown by the broken lines and the means of the selected parents by solid lines. The differences between rapid and slow lines are shown in parenthesis.

that no control was run, and the results are summarized in Figure 8. A thermostat failure terminated the experiment at generation 5.

Although the variance of the F_2 (Table II) was greater than that of the outbred stock already described, progress under selection was very much less. An estimate of the realized heritability[3] gave a value of 8.9 percent. This low estimate was obviously due to the failure to increase the rate of development by selection. The response of the "slow line" corresponds to a realized heritability of about 25 percent if it is assumed that the starting population (F_2) can be taken as a control level for each subsequent generation. That is to say, in the second experiment response to selection behaves as in the first trial, *but only in one direction:* there is no progress in the direction of more rapid larval development and some indication of the converse.

The nature of the F_2 which gave this anomalous result was next examined, together with the parents, F_1 and backcrosses. The results are summarized in Figure 9, and in Table II. It is obvious that the parental lines are quite different, the Ore K showing a much greater variability than the Edinburgh Large. Secondly, the F_1 shows a notable degree of heterosis which makes any conventional analysis[7] impossible. On the logarithmic scale the variance of this F_1 is the same as that of the Edinburgh Large line, implying that Ore K is more susceptible to environmental differences than either of these, and hence that the two parental genotypes react differently with the environment. Thirdly, while the mean of the F_2 is close to expectation its variance is less than that of Ore K and its distribution of pupation times (Figure 9) corresponds almost exactly with that of the backcross to the Ore K line. This backcross develops faster than expected, whereas the backcross to Edinburgh Large is slower than expected, and behaves almost exactly like the Edinburgh Large line itself. Mean development times thus rank as follows: $F_1 < B_2 \sim F_2 \sim B_1 \sim P_1 < P_2$, differences between B_2

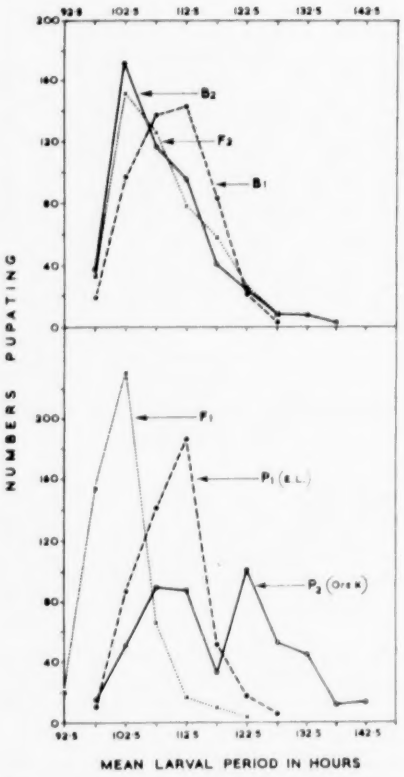
TABLE II. Pupation time and larval survival of the pure lines and crosses*

Genotype	E. L. (P_1)	Ore K (P_2)	F_1	F_2	EL × F_1 (B_1)	Ore K × F_1 (B_2)
Larval development time (hours)						
mean	110.12	122.25	101.96	109.33	109.94	108.27
variance (s^2)	33.52	120.00	25.35	68.11	37.39	58.28
number	167	257	470	411	242	201
Larval development time (log. days)						
mean	0.661	0.705	0.627	0.654	0.660	0.653
variance (s^2)	0.00048	0.00142	0.00045	0.00076	0.00058	0.00095
Percent pupae formed	83.5	73.5	94.0	82.1	80.7	80.4

*Since all matings were tested concurrently the numbers set up vary with the genotype. As before, larvae were set up 50 per vial.

to B_1 are small and not significant. While throwing some light on the problem noted in the preceding paragraph, this test raises further issues which are now being studied using different strains and other environmental conditions outside the scope of this paper.

One important difference between these matings is larval survival (percent pupation in Table II) which is not the same for all genotypes. Again the two parent lines differ, the F_1 shows hybrid vigor in this character, and there is a negative correlation between survival and development time. However, in the case of the F_2 and backcrosses survival is lower than would be predicted from the behavior of the homogeneous lines. This is particularly striking for the Edinburgh Large backcross where only 80.7 percent of larvae survive instead of the expected 88.5 percent, ($\chi^2 = 6.79$, d.f. 1, $p < 0.01$). Previous work[14] suggests that early larval mortality should lead to an improvement of development times by reducing the effects of crowding, but this is obviously not the case for the Edinburgh Large backcross. The situation with respect to development rate cannot be explained, therefore, in simple terms of crowding and competition, and we must conclude that the different genotypes may not only respond differently to the environment but may also interact with each other in some complex way as they grow. These two factors could cause the abnormal F_2 and backcross distributions.



DEVELOPMENT TIME OF EDINBURGH LARGE AND ORE K

Figure 9

The distributions of development times of the Edinburgh Large and Ore K populations and of their F_1, F_2 and Backcrosses. Note that the total number in each population has been adjusted to 500 to simplify comparisons. All crosses were made reciprocally.

Discussion

Strong[16] has noted a number of examples in mice where progress is in the direction opposite to selection pressure, such as age at first litter, litter size and spacing, latent period to induced neoplasia etc. Such composite characters generally show hybrid vigor. In other recorded cases there may be little or no progress in one direction while selection is successful in the opposite direction[4]. Or, more commonly the response to two-way selection may be asymmetrical (see Falconer[3]), progress being more rapid one way than the other. An example, which is particularly relevant to the results summarized in Figure 8, is rate of growth in swine[5]. There selection is effective in the direction of slower growth, but only to a small extent, if at all, towards faster growth. And this is again a complex character which shows hybrid vigor in suitable crosses. The unidirectional response also leads to a low estimate of heritability for this character (taken as weight at 180 days) at 18.6 percent when computed from the results of two-way selection, as compared with other possible estimates of 23.9 percent (from variance within and between litters) and of 65 percent (calculated from offspring-dam regression). So this example parallels the one recorded above to a surprising degree, although it does not

follow that the causes are the same in the two instances.

Comparison of the pure lines and their crosses (Table II) suggests that two tendencies affect length of larval development:

(1) the presence of some genes in the P_2 (Ore K) which when homozygous in a suitable background reduce development time,

(2) the general level of heterosis.

The first relationship is possible since the normal procedure for keeping pure lines (P_2) would encourage the selection of early emerging virgin flies. Conversely, selection for large size (P_1) tends to increase development time[11]. Both effects are indicated by the behavior of the backcrosses, though the former seems the more significant in this instance. The overriding importance of heterosis in this character is indicated by the similarity of the distributions of the F_2 and backcrosses which are each heterozygous for 50 percent of loci. However, none of these second generation crosses produce larvae which develop as fast as the best F_1 time, probably because most, if not all, individuals will carry some loci homozygous for P_1 and P_2 genes, but possibly also due to some interaction between the developing larvae which affects their survival (Table II). The similarity of the B_2 and B_1 mean development times further suggests that the slow development of the P_2 is due to the interaction together of a number of homozygous loci, and that genotypes with this necessary combination rarely turn up in the B_2. A similar effect has been described by Tantawy and Reeve[17] working on the effects of inbreeding on size and survival in *Drosophila*.

Since selection for rapid development in the *Drosophila* experiment was based on the fastest growing fifth of the F_2 population, the selected group will be made up largely of heterozygous individuals, and with some P_2 rather than P_1 genes homozygous. Consequently the selected group will produce a further segregating population like the F_2, but biased towards the backcrosses to an extent which cannot be easily defined. Subsequent selection will necessarily continue on this pattern giving a trend opposite to the direction of selection, at least during the first generations. In this instance, the result is a direct consequence of the heterotic nature of the character and of the system of selection employed. Conversely, selection for slow growth will generally pick out types like the parents, and progress is possible from reassortment among these. From published data it is difficult to be sure how far a similar situation (i.e. selection of heterozygotes which give the most extreme manifestation of the character) applies in the cases quoted above, but Krider *et al.*[5] note that their work started from a population of diverse origins.

Falconer[4] has noted that heritability estimates based on two-way selection are misleading when progress is made only in one direction, and corrects his estimate for lactation performance in mice accordingly. What is perhaps more important is to point out that heritability estimates as conventionally computed will necessarily overestimate the possibilities of progress when selection is in the direction in which hybrid vigor is displayed. This will be particularly true when the parental stock is a cross of lines or varieties.

Summary

The heritability of "rate of larval development" in *Drosophila* was found to be between 20 and 25 percent when estimated from the results of two-way selection in an outbred population. However, two-way selection from an F_2 of two inbred lines led to progress only in the direction of slower growth. The character, like many of economic importance, shows hybrid vigor, so that selection for rapid growth is necessarily mainly selection of heterozygotes, and progress in this direction tends to be ineffective or even negative. Examination of F_2 and backcross generations suggests that the situation is further complicated by the slow growth of one parent being due to an interaction between a number of homozygous loci. There may also be some ecological interaction between larvae of different genotypes as they develop together.

Literature Cited

1. Bell, A. E., C. H. Moore and D. C. Warren. A biological evaluation with *Drosophila melanogaster* of four methods of selection for the improvement of quantitative characteristics. *Caryologia* 6 (suppl.): 851-853. 1954.

2. Clayton, G. A., J. A. Morris and A. Robertson. An experimental check on quantitative genetical theory. *Jour. Genet* 55: 131-151. 1957.

3. Falconer, D. S. Asymmetrical response in selection experiments. *I.U.B.S. Symposium on Genetics of Population Structure, Pavia.* 1953.

4. Falconer, D. S. Patterns of response in selection experiments with mice. *Cold Spring Harbor Symposia on Quant. Biol.* 20: 178-196. 1955.

5. Krider, J. L., B. W. Fairbanks, W. E. Carroll and E. Roberts. Effectiveness of selecting for rapid and for slow growth rate in Hampshire swine. *Jour. Animal Sci.* 5: 3-15. 1946.

6. Mather, K. Variation and selection of polygenic characters. *Jour. Genet.* 41: 159-193. 1941.

7. Mather, K. Biometrical Genetics. Methuen, London. pp. 162. 1949.

8. Pittendrigh, C. S. On temperature independence in the clock system controlling emergence time in *Drosophila*. *Proc. Nat.*

Acad. Sci. (Wash.), 40: 1018-1029. 1954.

9. Powsner, L. The effects of temperature on the duration of the developmental stages of *Drosophila melanogaster*. *Physiol. Zool.* 8: 474-520. 1935.

10. Rasmuson, M. Selection for bristle numbers in some unrelated strains of *Drosophila melanogaster*. *Acta Zool.* 36: 1-49. 1955.

11. Reeve, E. C. R. Natural selection for body size in *Drosophila*. *Caryologia* 6 (suppl.): 854-855. 1954.

12. Robertson, F. W. and J. H. Sang. Ecological determinants of population growth in a *Drosophila* culture. I. Fecundity of adult flies. *Proc. Roy. Soc.* B. 132: 258-277. 1941.

13. Robertson, F. W. and E. Reeve. Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. *Jour. Genet.* 50: 414-448. 1952.

14. Sang, J. H. Ecological determinants of population growth in a *Drosophila* culture. III. Larval and pupal survival. *Physiol. Zool.* 22: 183-202. 1949.

15. Sang, J. H. The quantitative nutritional requirements of *Drosophila melanogaster*. *Jour. Exp. Biol.* 33: 45-72. 1956.

16. Strong, L. C. Biological equilibrium and the origin of cancer. *Science* 125: 595-596. 1957.

17. Tantawy, A. O. and E. C. R. Reeve. Studies in quantitative inheritance. IX. The effects of inbreeding at different rates in *Drosophila melanogaster*. *Z. Vererbungslehre* 87: 648-667. 1956.

POLLEN CYTOLOGY AND SELF-INCOMPATIBILITY SYSTEMS IN PLANTS

JAMES L. BREWBAKER*

THERE are two types of pollen grains in the angiosperms, cytologically speaking. One is *binucleate*—the other, *trinucleate*. Binucleate grains contain a generative cell and a vegetative or tube nucleus, the generative cell dividing during pollen tube growth to form the two male gametes. A trinucleate grain is formed when the division of the generative cell occurs before the pollen is shed.

There are two types of self-incompatibility in homomorphic angiosperms, genetically speaking. One is the *gametophytic* type—the other, *sporophytic*. Both types are based on the action and interaction of multiple alleles of a single *S* locus (rarely, of two loci). In the gametophytic system pollen behavior (i.e., function or failure in a particular mating) is determined gametophytically by the *S* allele in each grain. The incompatibility inhibition regularly occurs at some stage during pollen tube growth in the pistil of gametophytic species. In the sporophytic system pollen behavior is sporophytically determined (imposed by the maternal genotype), and inhibition commonly occurs at the stigma surface so as to inhibit pollen germination or drastically curtail pollen tube growth.

It is observed here that these phenomena are quite regularly associated, the gametophytic system with binucleate grains and the sporophytic with trinucleate grains.

Self-Incompatibility

Among the several schemes which promote or enforce outbreeding in plants, that of self-incompatibility appears to have played a major role in the evolution of flowering plants. Self-incompatibility is the inability of a plant producing functional male and female gametes to set seed when self-pollinated. Special attention is given in this paper to homomorphic incompatibility, in distinction to heteromorphic species in which differences in floral morphology are associated with incompatibility. Nearly half of the world's major crop and ornamental species represent genera in which self-incompatibility is known to occur. Self-incompatible species have been found in at least 66 plant families, which are distributed throughout the angiosperms and occur in every major phylogenetic line[5, 12].

It is rather more convenient to ascribe this distribution to the independent origin of various self-incompatibility systems at different points in evolutionary history. The opposing theory—that of a single point of origin—implies, in essence, that the majority (if not all) of angiosperms trace back to a single, self-sterile plant[38]. The second theory derives much support from a steadily increasing body of genetic data which indicates a remarkable similarity in the hereditary basis of self-incompatibility in homomorphic flowering plants. This genetic system, which has been described with supporting data from at least 33 species representing 11 different plant families, is that of a single *S* gene locus (rarely of two loci) with multiple alleles; the physiological result of gene action being the inhibition of pollen germination or of pollen-tube growth in an incompatible mating.

Types of Incompatibility Systems

Two types of incompatibility systems, both constructed on the single gene, multiple allele foundation have been described for homomorphic plants. The

*Brookhaven National Laboratory, Upton, L. I., New York. Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

gametophytic system was proposed by Prell[32] with the first supporting data obtained by East and Mangelsdorf[6,7] in *Nicotiana* hybrids and by Lehman[22] in *Veronica*. Incompatibility results when the *S* allele in a pollen grain is also present in the pistil. Thus the incompatibility is determined "gametophytically" by the particular allele in the pollen grain. The inhibition in gametophytic species regularly occurs at some stage during pollen tube growth in the pistil, although partial inhibition of pollen germination is known to occur. The 21 species reported to have the gametophytic system (Table I) are distributed in seven plant families (this excludes the grasses, to be considered in detail later). All of these plants are insect-pollinated, most of them having conspicuous inflorescences with copious stigmatic fluid (which may not appear until tripping, as in some legumes). Dominance among gametophytic *S* alleles cannot occur in haploid pollen and is not known to occur in the diploid style, where it would lead to self-fertility of a readily recognized type. In autotetraploids, however, *S* allele interactions do occur in the heterogenic diploid pollen grain. One of these is the competition interaction ("mutual weakening"), of fairly rare occurrence, in which neither allele is fully active, with the result that such grains are not inhibited (or are only partially inhibited in *Oenothera*, in which the broad blunt tubes literally have to bore their way through pistillate tissue[28]). The second interaction observed in tetraploids is that of dominance, in which one allele partially or completely suppresses the action of the other allele in the heterogenic pollen.

The second type of incompatibility system — the sporophytic system — was first described with supporting data by Gerstel[14] and by Hughes and Babcock[17]. It has been reported subsequently for a

TABLE I. Pollen cytology and site of inhibition for homomorphic species of known incompatibility genetics

Family	Species	Type of *S* system	Type of pollen	Site of inhibition
Commelinaceae	*Tradescantia virginiana*	G	bi	style
Leguminosae	*Trifolium* (4 spp.)	G	bi	style
Onagraceae	*Oenothera* (2 spp.)	G	bi	style
Papaveraceae	*Papaver nudicaule*	G	bi	ovary*
Rosaceae	*Prunus avium*	G	bi	style
	Pyrus communis	G	bi	style
Scrophulariaceae	*Antirrhinum glutinosum*	G	bi	style
	Nemesia strumosa	G	bi	style
	Verbascum phoeniceum	G‡	bi	—
	Veronica syriaca	G	bi	—
Solanaceae	*Lycopersicon peruvianum*	G	bi	style
	Nicotiana (2 spp.)	G	bi	style
	Petunia (3 spp.)	G	bi	style
	Solanum spp.	G	bi	style
Compositae	*Cosmos bipinnatus*	S	tri	stigma
	Crepis (2 spp.)	S	tri	stigma
	Parthenium argentatum	S	tri	stigma
Cruciferae	*Brassica oleracea*	S	tri	stigma
	Capsella grandiflora	S	tri	stigma
	Iberis amara	S	tri	stigma
	Lesquerella densipila	S	tri	stigma
	Raphanus sativus	S	tri	stigma
Sterculiaceae	*Theobroma cacao*	S	—	ovary†
Gramineae	*Phalaris coerulescens*	G‡	tri	style
	Secale cereale	G‡	tri	style

* no style
† hollow style
‡ two loci

G = Gametophytic type
S = Sporophytic type

bi = binucleate
tri = trinucleate

number of composites and crucifers, as well as for *Theobroma cacao*. In the sporophytic system pollen reaction is controlled sporophytically; i.e., it is imposed by the maternal genotype. As a result, all pollen grains from a given plant behave similarly. For example, both pollen types S_1 and S_2 from plant S_1S_2 will behave as S_1 if S_1 is dominant to S_2, or as S_2 if S_2 is dominant, or as S_1 plus S_2 if there is no dominance. There is also some evidence for dominance in the pistil in plants having the sporophytic system. Inhibition regularly occurs at the stigmatic surface, resulting in the inhibition of pollen germination (one exception being *Theobroma cacao*). The 10 species believed to have this system are distributed in three families (Table I).

While the gametophytic and sporophytic systems appear at first glance to be quite dissimilar, they can be interpreted to differ in only one major respect, namely, *the time of S gene action in microsporogenesis*. In the gametophytic system, the *S* allele appears to go into action to produce the inhibitory factor or its precursor at or following anaphase of the first meiotic division of the microsporocytes. In the sporophytic system, the *S* allele action must occur before anaphase I. In the gametophytic case, a quartet of spores thus may consist of two biochemically different pairs of spores, whereas in the sporophytic case all spores in the same anther will be phenotypically identical.

Cytology of the Pollen Grain

The mature pollen grains of angiosperms are either binucleate or trinucleate, a fact upon which Schnarf[36] focused attention. In a study involving 143 species of flowering plants, Schnarf reported 41 families with binucleate species, nine with trinucleate and nine in which both types were found. From a similar study of 147 species in this lab, and from other published information (cf. Maheshwari[28]), it is evident that at least 67 families (as of Lawrence[21]) are binucleate, 28 are trinucleate and 13 contain both types. The phylogenetic distribution of these families definitely implicates the advanced or derived position of the trinucleate condition.

Considering only those families in which self-incompatibility is known to occur, the following distribution is observed:

Binucleate: Acanthaceae, Amaryllidaceae, Anonaceae, Begoniaceae, Betulaceae, Bignoniaceae, Bromeliaceae, Commelinaceae, Ericaceae, Euphorbiaceae, Fagaceae, Iridaceae, Leguminoseae, Liliaceae, Loganiaceae, Lythraceae, Moraceae, Nymphaeaceae, Onagraceae, Orchidaceae, Papaveraceae, Primulaceae, Resedaceae, Rosaceae, Saxifragaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Tropaeolaceae, Verbenaceae, Violaceae, Zingiberaceae.

Trinucleate: Apocynaceae, Caprifoliaceae, Compositae, Cruciferae, Geraniaceae, Gramineae, Malvaceae, Nyctaginaceae, Plantaginaceae, Plumbaginaceae, Polygonaceae, Portulacaceae.

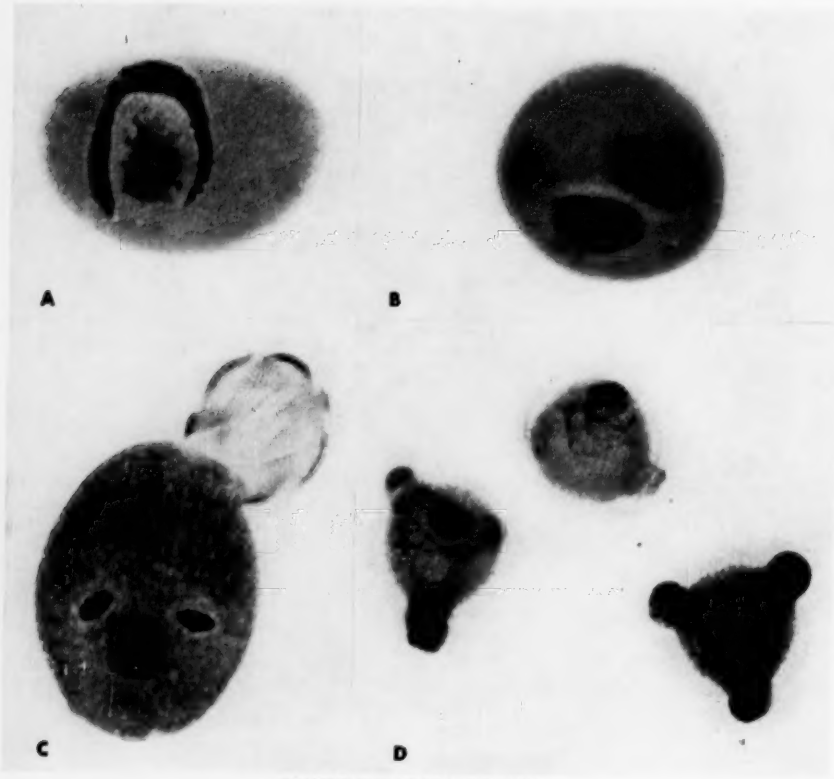
Both: Asclepiadaceae, Boraginaceae, Campanulaceae, Convolvulaceae, Labiatae, Oleaceae, Polemoniaceae, Ranunculaceae, Rubiaceae, Rutaceae.

Unknown: Aceraceae, Cistaceae, Cornaceae, Dioscoreaceae, Goodeniaceae, Hamamelidaceae, Lentibulariaceae, Myrtaceae, Passifloraceae, Thymeleaceae.

Binucleate grains contain a generative cell and a vegetative or pollen-tube nucleus. This unique cell within a cell situation is referred to by some authors as bicellular[28]. The generative cell may be spindle-formed, as in *Tradescantia virginiana* (Figure 10*A*) or relatively spherical as in the liliaceous *Smilacina stellata* (Figure 10*B*), and commonly stains quite deeply with aceto-carmine. The cell wall and scanty cytoplasm of the generative cell show clearly in special preparations. The vegetative nucleus regularly appears to have rather diffuse chromatin and to be degenerative; in many species it does not appear at all in mature grains ("unicellar" grains). In a few instances, trinucleate grains have been observed in binucleate species (e.g., *Iris*, *Gasteria*).

Trinucleate (or "tricellular") grains contain a vegetative nucleus and the two male gamete cells, the generative cell having divided prior to maturation of the grains. The two gametes range from spherical as in the crucifer, *Physaria floribunda* (Figure 10*C*), to the thin laminae of a composite such as *Chrysanthemum leucanthemum* (Figure 10*D*). As in binucleate grains, the vegetative nucleus seldom stains intensely.

In at least one family, the Labiatae, the pollen cytology is correlated with the external morphology of the grain: 3-colpate grains (having three furrows) are binucleate while 6-colpate grains are trinucleate (Erdtman[10]). It is evident that the number of furrows may be determined by trischistoclasia at quartet stage[39] suggesting that the occurrence of the first mitotic division during quartet stage might produce 6-colpate grains, while microspores in which the first division occurs after the spores separate would become 3-colpate.



MATURE POLLEN GRAINS

Figure 10

Mature pollen grain of *A—Tradescantia virginiana; B—Smilacina stellata; C—Physaria floribunda;* and *D—Chrysanthemum leucanthemum.*

The bi- and tri-nucleate conditions may be considered to differ in one major respect, namely, *the time of division of the generative cell.* This occurs relatively late in binucleate grains, relatively early in trinucleate. Similarly, the 3- and 6-colpate conditions, at least in the mint family, may represent late and early divisions, respectively, of the microspore nucleus.

The Correlation

A consideration of the 33 species for which the genetics of incompatibility is fairly well established makes apparent the general association of binucleate grains with stylar inhibition and the gametophytic system, and of trinucleate grains with stigmatic inhibition and the sporophytic system (Table I). In several additional species for which the genetics of incompatibility is not known, the correlation of pollen cytology and site of inhibition is similarly evident (Table II).

A significant exception to this correlation occurs in the grass family (Table I). *Secale cereale* is known from the extensive studies of Lundqvist[25, 27] to have two *S* loci, as found also by Hayman[16] in *Phalaris coerulescens* and in *Festuca pratensis* by Lundqvist[26]. Whether both loci behave in the gametophytic or sporophytic fashion is not readily determined, although both authors favor gametophytic control. However, all grasses are trinucleate and inhibition regularly curtails tube growth to the tips of stylar hairs[16].

Several examples are cited in Tables I and II in which inhibition occurs in the ovary. This phenomenon has been described in some detail by Sears[30] for *Gasteria verrucosa,* by Stout and Chandler[37] for *Hemerocallis citrina,* by Brock[2] for *Lilium* spp. and by Giles[15] for *Lotus corniculatus.* In each of these species occasional selfed tubes penetrate the megaga-

metophyte. Other examples are *Reseda* spp.[9] and *Papaver nudicaule*. Each of these species is bicellular and each is hollow-styled or lacks a style altogether (as *Reseda*). It would appear that a certain intimacy of tube and pistillate tissue is required for inhibition.

One exception to the inhibition of sporophytic type pollen at the stigma surface would appear to be *Theobroma cacao*, a species interpreted by Knight and Rogers[19] to have the sporophytic system. Whether the hollow-styled *Theobroma* is bi- or trinucleate seems not to have been recorded, although *Hermannia disticha* of the Sterculiaceae is binucleate[34]. The data summarized by Knight and Rogers could not be interpreted readily on a gametophytic scheme.

Another species of interest is the sugar beet, *Beta vulgaris*, in which the only pertinent incompatibility data were interpreted as suggesting two gene loci with gametophytic control[31]. However, the data might also be interpreted on the sporophytic scheme. *Beta* has trinucleate pollen, although Savitsky[33] found that pollen germination in compatible and incompatible matings was quite comparable, the inhibition inferred to occur in the pistil (which has a short hollow stylar canal[1]).

Several species with heteromorphic incompatibility also show the correlation of pollen cytology and site of inhibition. Schoch-Bodmer[35] found that the binucleate *Primula* and *Lythrum* spp. showed inhibition in the style, while inhibition of the trinucleate grains of *Linum* and *Fagopyrum* spp. occurred at the stigma surface. Lewis[23] observed disparity in osmotic pressures of "pin" and "thrum" pollen of *Linum grandiflorum*, but noted the inadequacy of this as the sole explanation for incompatibility inhibition.

The genetic basis for heteromorphic incompatibility is an incompatibility locus with only two alleles, generally linked closely with genes affecting style and anther length, among other characters. It appears likely that this locus evolved independently of the *S* locus (or loci) of homomorphic species, lacking as it does the allelic plasticity of the latter gene. Thus the maternal determination of pollen behavior of the *Ss* heterozygote may have a morphogenetic basis different from that of sporophytic homomorphic incompatibility.

The observed correlation is suggestively a function of the relative timing of a series of events in microsporogenesis:

System	Time of *S* gene action in microsporogenesis	Time of incompatibility inhibition	Time of generative cell division
Gametophytic—binucleate	Late—At or after meiosis	Late—During tube growth	Late—During tube growth
Sporophytic—trinucleate	Early—Before meiosis	Early—At time of germination	Early—Before pollen is shed

It might be argued that a single major change in timing of these (related?) events during sporogenesis and male gametogenesis led to the evolution of the sporophytic-trinucleate from the gametophytic-binucleate system.

Discussion

Sucrose appears to be a key to successful germination and growth of pollen *in vivo*. Optimum germination and growth *in vitro* seldom obtain in the absence of sucrose and of boric acid, the latter of probable significance as a sugar-transport agent[13]. While a primary role of sucrose is clearly an osmotic one, its importance as a metabolite deserves attention. Linskens[24] has demonstrated that compatible tubes metabolize all available sucrose in petunia styles, whereas the sucrose is not used up in an incompatible mating (although incompatible tubes penetrate the style several mm.). O'Kelley[32] has shown that pollen tubes metabolize a high proportion of available sucrose in culture media.

It is tempting to propose that the physiological action of incompatibility alleles may be mediated simply via the control of sucrose uptake or metabolism by the pollen grain or tube. It is probable that the immediate *S* gene products are of antigen-antibody type, interacting immunity-fashion to thus disrupt growth.

It appears possible that trinucleate grains might be relatively sucrose- or metabolite-deficient at maturity due to the occurrence of the second mitotic division in late microsporogenesis. In contrast, binucleate grains might be relatively metabolite-rich when shed. Restriction of the availability of sucrose to a metabolite-deficient trinucleate grain would thus result in the inhibition of its germination. Under similar circumstances binucleate grains would germinate, but tube growth would cease when available metabolites in the grain had been utilized.

Observations that may be related to the suspected metabolite-concentration difference include the fact that a higher proportion of trinucleate plants are successfully anemophilous. Of similar interest is the observation that germination of most binucleate pollen may be accomplished readily in 3-10 percent sugar or sugar-agar solutions, especially upon addition of 10-100 ppm. boric acid. However, trinucleate grains seldom germinate satisfactorily when submerged or floating in such so-

lutions, and generally require higher sugar concentrations. The inferred turgor pressure relationships could be linked to physiological changes occurring during second mitosis.

It has been demonstrated recently by Brewbaker and Majumder (unpublished) that pollen tube growth of self-incompatible petunias may be inhibited drastically *in vitro* by stylar H_2O-extracts of the same plants. This corroborates the observations of Yasuda[40] on petunia, Coutaud[4] on apple, and East and Park[8] on *Nicotiana* sp. (although not always so interpreted) that the *S* allele inhibitory substance of the pistil is active in water extracts. It remains to be seen whether the germination and early growth of tricellular grains may be similarly inhibited *in vitro*.

Conclusion

A correlation is observed relating pollen cytology, type of incompatibility system, and site of incompatibility inhibition in homomorphic flowering plants. Gametophytic type incompatibility is observed primarily in species with binucleate pollen and with inhibition occurring during pollen tube growth. Sporophytic incompatibility is linked to trinucleate grains and inhibition of germination or early tube growth. The most conspicuous exceptions to the correlation are members of the Gramineae.

An almost perfect association of pollen anatomy and site of inhibition (binucleate inhibited in style or ovary, trinucleate on stigma) exists in 42 genera from 23 different families. It is suggested that this association might be the result of a difference in the amount of available metabolites in the bi- vs. the tri-nucleate grains at anthesis, with incompatibility acting to restrict sucrose uptake or metabolism by the male gametophyte.

The inhibition of pollen-tube growth *in vitro* by self-stylar extracts is demonstrated for petunia.

Literature Cited

1. Artschwager, E. Micro and macrosporogenesis in sugar beet with special reference to the problem of incompatibility. *Mem. Hort. Soc. N. Y.* 3: 295-297. 1927.
2. Brock, R. D. Fertility in Lilium hybrids. *Heredity* 8: 409-420. 1954.
3. Cooper, J. R. The behavior of pollen tubes in self- and cross-pollination. *Proc. Amer. Soc. Hort. Sci.* 25: 138-140. 1928.
4. Coutaud, J. Influence des stigmates sur la qualite germinative de differentes varieties de Pommier. *Compt. Rend. Acad. Sci.* 226: 2090-2091. 1948.
5. East, E. M. The distribution of self-sterility in the flowering plants. *Proc. Amer. Phil. Soc.* 82: 449-518. 1940.
6. ———— and A. J. Mangelsdorf. A new interpretation of the hereditary behavior of self-sterile plants. *Proc. Nat. Acad. Sci.* 11: 166-171. 1925.
7. ————. Studies on self-sterility. VII. Heredity and selective pollen tube growth. *Genetics* 11: 466-481. 1926.
8. ———— and J. B. Park. Studies on self-sterility. II. Pollen-tube growth. *Genetics* 3: 353-366. 1918.
9. Eigsti, O. J. Pollen tube behavior in self-fertile, self-sterile and interspecific pollinated Resedaceae. *Amer. Nat.* 71: 520-521. 1937.
10. Erdtman, G. Pollen Morphology and Plant Taxonomy. Almqvist and Miksells, Upsala, Sweden. 1952.
11. Fagerlind, F. Embryologische, zytologische und bestaubungsexperimentelle Studien

TABLE II. Pollen anatomy and site of inhibition in self-incompatible species for which genetics is not known

Family	Self-incompatible species	Type of pollen	Site of inhibition	Authority
Euphorbiaceae	*Euphorbia splendens*	bi	ovary	East[5]
Fagaceae	*Castanea* sp.	bi	ovary	McKay[29]
Leguminosae	*Lotus corniculatus*	bi	ovary*	Giles[15]
Liliaceae	*Gasteria* sp.	bi	ovary*	Sears[30]
	Hemerocallis spp.	bi	ovary*	Stout & Chandler[37]
	Lilium spp.	bi	ovary*	Brock[2]
Papaveraceae	*Corydalis cava*	bi	style	Jost[18]
Resedaceae	*Reseda odorata*	bi	ovary†	Eigsti[9]
Rosaceae	*Malus* sp.	bi	style	Cooper[3]
Rubiaceae	*Coffea* sp.	bi	style	Fagerlind[11]
Saxifragaceae	*Tolmiea menziesii*	bi	style	Sears[30]
Convolvulaceae	*Ipomoea batatas*	tri	stigma	Kogari *et al*[20]
Geraniaceae	*Pelargonium hort.*	tri	stigma	Sears[30]
Labiatae	*Rosmarinus officinalis*	tri	stigma	East[5]
	Salvia spp.	tri	stigma	East[5]

* hollow style
† no style

in der Familie Rubiaceae nebst Bemerkungen uber einige Polyploiditats-probleme. *Acta Horta Bergiani* 11: 195-470. 1937.

12. FRYXELL, P. A. Mode of reproduction of higher plants. *Bot. Rev.* 23: 135-233. 1957.

13. GAUCH, H. G. and W. M. DUGGER. The role of boron in the translocation of sucrose. *Plant Phys.* 28: 457-466. 1953.

14. GERSTEL, D. U. Self-incompatibility studies in guayule. II. Inheritance. *Genetics* 35: 482-506. 1950.

15. GILES, W. L. The morphological aspects of self-sterility in *Lotus corniculatus* L. Ph.D Thesis, U. of Missouri. 1949.

16. HAYMAN, D. L. The genetical control of incompatibility in *Phalaris coerulescens* Desf. *Austr. Jour. Biol. Sci.* 9: 321-331. 1956.

17. HUGHES, M. R. and E. B. BABCOCK. Self-incompatibility in *Crepis foetida* (L.) subsp. *rhoedifolia* (Bieb.) Schinz et Keller. *Genetics* 35: 570-588. 1950.

18. JOST, L. Uber die Selbststerilitat einiger Bluten. *Bot. Ztg.* 65: 77-117. 1907.

19. KNIGHT, R. and H. H. ROGERS. Incompatibility in *Theobroma cacao*. *Heredity* 9: 69-77. 1955.

20. KOGARI, Y. and U. KAWAHARA. Studies on the self- and cross-incompatibility in sweet potato. *Imp. Agr. Exp. Sta. Bull.* 52, Tokyo. 1942.

21. LAWRENCE, W. J. C. Taxonomy of Vascular Plants. MacMillan, New York. 1951.

22. LEHMANN, E. The heredity of self-sterility in *Veronica syriaca*. *Mem. Hort. Soc. N. Y.* 3: 313-320. 1926.

23. LEWIS, D. The physiology of incompatibility in plants. II. *Linum grandiflorum*. *Ann. Bot.* 7: 115-122. 1943.

24. LINSKENS, H. Physiologische Untersuchungen der Pollenschlauch-Hemmung selbsteriler Petunien. *Zeit. f. Botanik* 43: 1-44. 1955.

25. LUNDQVIST, A. Studies on self-sterility in rye, *Secale cereale* L. *Hereditas* 40: 278-294. 1954.

26. ———. Genetics of self-incompatibility in *Festuca pratensis* Huds. *Hereditas* 41: 518-520. 1955.

27. ———. Self-incompatibility in rye. I. Genetic control in the diploid. *Hereditas* 42: 293-348. 1956.

28. MAHESHWARI, P. The male gametophyte of Angiosperms. *Bot. Rev.* 15: 1-75. 1949.

29. MCKAY, J. W. Self-sterility in the Chinese chestnut (*Castanea mollissima*). *Proc. Amer. Soc. Hort. Sci.* 4: 156-160. 1942.

30. O'KELLEY, J. C. External carbohydrates in growth and respiration of pollen tubes in vitro. *Amer. Jour. Bot.* 42: 322-327. 1955.

31. OWEN, F. V. Inheritance of cross- and self-sterility and self-fertility in *Beta vulgaris*. *Jour. Agric. Res.* 64: 679-698. 1942.

32. PRELL, H. Das Problem der Unbefruchtbarkeit. *Naturwiss. Wochenschr.* N. F. 20: 440-446. 1921.

33. SAVITSKY, H. A method for determining self-sterility and self-fertility in sugar beets based upon the stage of ovule development shortly after flowering. *Proc. Amer Soc. Sugar Beet Tech.* 1950: 198-201. 1950.

34. SCHNARF, K. Studien uber den Bau der Pollenkorner der Angiosperm. *Planta* 27: 450-465. 1937.

35. SCHOCH-BODMER, H. Zur Frage der "Hemmungsstoffe" bei Heterostylen. *Arch. Julius Klaus-Stift.* 20: 403-416. 1945.

36. SEARS, E. R. Cytological phenomena connected with self-sterility in the flowering plants. *Genetics* 22: 130-181. 1937.

37. STOUT, A. B., and CLYDE CHANDLER. Pollen-tube behavior in Hemerocallis with special reference to incompatibilities. *Bull. Torrey Bot. Club.* 60: 397-416. 1933.

38. WHITEHOUSE, H. L. K. Multiple-allelomorph incompatibility of pollen and style in the evolution of the Angiosperms. *Ann. Bot.* 14: 199-216. 1950.

39. WODEHOUSE, R. P. Pollen Grains. McGraw-Hill Book Co., New York. 1935.

40. YASUDA, S. Physiological research on self-incompatible plants of *Petunia violacea*. *Bull. Imp. Coll. Agr. and For. (Morioka)* 20: 1-95. 1934.

◆

CORRIGENDUM

In V. 48(3), Mid-South Oat Variety, p. 104, Figure 4 (Crown-Rust Reaction on Oat Leaves), *D-F* should read: Crown-Rust reaction in the greenhouse at juvenile stage, artificial inoculation with races 45 and 57 on *Victorgrain 48-93*, *Mid-South*, and *susceptible oat*, respectively.

—S. S. IVANOFF

A SELF-COMPATIBLE HYBRID FROM A CROSS BETWEEN TWO SELF-INCOMPATIBLE SPECIES IN TRIFOLIUM

KAMLA K. PANDEY*

INTERSPECIFIC hybridization in the genus *Trifolium* has been largely unsuccessful[17,5,16,8]. However, sterile hybrid plants were obtained from the cross of *T. repens* ($2n = 32$) with *T. nigrescens* ($2n = 16$) by Trimble[16]. Keim[9], working on the same species as Trimble, got the same results. He also obtained hybrids of *T. ambigum* and *T. hybridum* utilizing an embryo culture technique. But these hybrids did not flower. Brewbaker and Keim[4] reported two fertile interspecific hybrids between induced autopolyploids of *T. repens* and *T. nigrescens*. The hybrid in which one of the autopolyploids, *T. repens* ($2n = 64$), was self-fertile, was self-fertile, but the other in which both parents were self-sterile, was self-sterile. The selfed progeny and the backcross tests showed that the *S* alleles in the two species were homologous and that the self-fertility in the hybrid was due to the competition between the *S* alleles in the diploid pollen.

Near absolute failure of interspecific hybridization in *Trifolium* is of great evolutionary interest. Species which are far removed taxonomically and very different in their morphology have very similar chromosome complexes, e.g., the European species, *T. glomeratum* and the Californian species, *T. obtusifolium*. On the other hand, we find closely related species with very different chromosome complexes. The wild red clover, *T. pratense*, is very similar to *T. medium*, but the former has 14 and the latter over 80 chromosomes. Furthermore, though the number is the same, the shape and size of the chromosomes may be different (viz., in *T. pratense* and *T. incarnatum*). The great variability in chromosome complexes in *Trifolium*, according to Wexelsen[17], is a result of mutational changes in species, which have become isolated by intersterility, rather than the result of hybridization. Thus, while early in the evolution of *Trifolium*, natural hybridization may have taken place[1,7], later mutational and chromosomal changes were dominant factors in the speciation of this genus. This resulted in the cytological incompatibility between the species which is responsible for the failure of most interspecific crosses in *Trifolium* today.

The two species involved in the present study have very different distributions. *T. repens* L. is a widely distributed species found in almost all regions of the world. *T. uniflorum* L. is strictly a local species distributed in the alpine regions of the Mediterranean area. A cross between these two morphologically and geographically different species affords an opportunity to study the evolution of the self-incompatibility gene in these two species.

Gametophytic multiple oppositional system (*Nicotiana* type) of self-incompatibility has been found to exist in four species of *Trifolium*. *T. repens*[2], *T. pratense*[18], *T. hybridum*[10] and *T. nigrescens*[3]. Self-incompatibility has not yet been investigated in *T. uniflorum* but probably the same system also occurs in this species.

Materials and Methods

Seeds of *T. repens* were obtained from the Welsh Plant Breeding Station,

*Publication 609, Department of Botany and Plant Pathology, Ohio State University, Columbus 10, Ohio (Present address) and John Innes Horticulture Institute, Hertford, England. The author is grateful to Dr. D. Lewis, F. R. S. for facilities and encouragement during the research and to The Graduate School, Ohio State University for the Mary S. Muellhaupt post-doctoral fellowship during the tenure of which this work has been written.

Aberystwyth. One clone of *T. uniflorum* was made available by Mr. J. Newell, Curator, John Innes Horticulture Institute, Hertford, England where this work was undertaken. The clones of *T. repens* and *T. uniflorum* used in these experiments being fully self- sterile, emasculation of flowers for cross-pollination was unnecessary. However, flowers in which stigma was uncontaminated by self-pollen were chosen for cross-pollination. Before pollination the anthers were removed from the flowers.

Results

From root tip smears, it has been ascertained that *T. uniflorum* has 32 ($2n$) chromosomes, the same as in *T. Repens*[17].

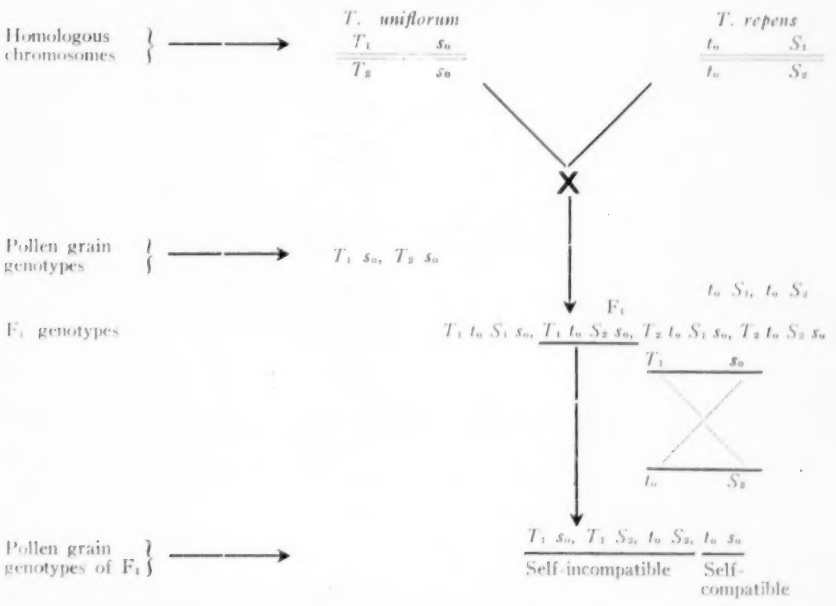
Interspecific crosses set 30-50 percent as many seeds as intra-specific crosses. However, out of 30 seeds sown only two (♀ *T. uniflorum* × ♂ *T. repens*) germinated. Of these, one was fully albino and died soon after germination, the other was three-fourths albino and had a sector of one-quarter green. With careful handling, this seedling survived, the green sector giving rise to a fully green shoot which developed into a healthy plant.

The morphological characters of the hybrid plant were intermediate of the two species. The size of the flower, size of the peduncle, number of flowers in a head all showed this phenomenon. However, the most remarkable result of the hybridization was that the hybrid was self-fertile. It had about 45 percent pollen fertility. The hybrid was selfed and reciprocally backcrossed to both the parents. The results in terms of crossability (+) and non-crossability (−) are as follows:

Hybrid selfed	+
Hybrid × *T. uniflorum*	+
Hybrid × *T. repens*	+
T. uniflorum × hybrid	+
T. repens × hybrid	+

These results could be explained on either of the following assumptions: (1) the *S* genes in the two species are located at different loci in the same homologous chromosome and there is considerable crossing-over between the two loci. (2) The *S* genes in the two species are located in different non-homologous chromosomes. The hybrid would thus produce four kinds of pollen grains, one of which will have none of the two *S* alleles and thus would be devoid of any self-incompatible reaction. This would lead to self-fertility. In reciprocal crosses with its parents half of the pollen grains in each cross would be compatible.

The result of the cross, if the *S* genes in the two species were loosely linked, are given below. For the sake of clarity, the *S* genes in *T. repens* are denoted by '*S*' series and those of *T. uniflorum* by '*T*' series. The recessive genes, s_o and t_o are of no significance.



The reactions of the hybrid in reciprocal crosses with its parents are shown in Table I. Exactly the same results would be obtained if the *S* genes were independent.

Discussion

Interspecific crosses in self-incompatible species in *Solanum* resulted in self-incompatible hybrids. Hybrids between two diploid species, *S. caldassii* and *S. subtillius*, resulted in four incompatibility groups in the F_1. This indicates that the alleles in the two species are homologous and that each series can work efficiently in a hybrid genetic background[13]. A similar relationship has been found in the hybrid between *S. rybinii* and *S. simplicifolium* by Black[10].

However, inter-series crosses, *S. aracc-papa* (Tuberosa) × *S. subtilius* (Commersoniana) revealed certain additional genes operating in the control of self- and cross-incompatibility in these species[14]. The results have been explained by Pushkarnath on the assumption that the normal homomorphic type of incompatibility system in *S. aracc-papa* is modified by another factor *R* which when present in the style, either in homozygous or heterozygous condition, prevents all pollen tubes carrying *S* alleles from effecting fertilization. According to him, "the presence of "*R*" gene in the pollen would appear to have no significance and it does not alter or influence the action of the basic *S* gene." However, a critical examination of his data indicates that the *R* gene in the male parent has the sporophytic effect of removing the specificity of the *S* alleles in the pollen. Thus in the cross ♀ $S_1\ S_2\ r_1\ r_1$ × ♂ $S_2\ S_{12}\ R_1\ r_1$, which should have normally produced four genotypes ($S_1 S_{12} R_1 r_1$, $S_1 S_{12} r_1 r_1$, $S_2 S_{12} R_1 r_1$, $S_1\ S_{12}\ r_1\ r_1$), eight genotypes ($S_1\ S_{12}\ R_1\ r_1$, $S_1 S_{12} r_1 r_1$, $S_2 S_{12} R_1 r_1$, $S_2 S_{12} r_1 r_1$, $S_1 S_2 R_1 r_1$, $S_1 S_2 r_1 r_1$, $S_2 S_2 R_1 r_1$, $S_2 S_2 r_1 r_1$) were recovered. This indicates that the pollen $S_2\ R_1$ and $S_2\ r_1$ which should have been incompatible on the female parent containing the S_2 allele, were compatible. Since both pollen grains, $S_1 R_1$ which contains *R* and $S_2\ r_1$ which lacks *R*, are compatible, the influence of *R* in removing the specificity of *S* alleles in pollen is sporophytically determined.

Another inter-series cross between *S. rybinii* (Tuberosa) and *S. subtilius* (Commersoniana) were reciprocally compatible[15]. All the progeny of the cross ♀ *S. rybinii* × ♂ *S. subtilius* were self-incompatible. They were cross-incompatible with each other and also reciprocally with both of their parents. These results are quite different from those observed in the other inter-series cross, *S. subtilius* × *S. aracc-papa*. Pushkarnath assumed that an independent system of *S* genes, other than those found in the series Commersoniana, was probably operating in the series Tuberosa. Further work on the self-incompatibility in these and other *Solanum* species should be rewarding.

Recent studies in the family Gramineae have shown that in *Secale cereale*, *Festuca pratensis*[11,12] and *Phalaris coerulescens*[6] the self-incompatibility is controlled gametophytically by two loci, each with a series of multiple alleles. Thus selection has had an opportunity here to work with allelic interaction in the pollen so as to render it consistent with a normal functioning of the incompatibility mechanism. In all other studied cases of self-incompatible angiosperms with homomorphic incompatibility, the incompatibility system is generally controlled by a single gene with a large number of alleles.

The present study reveals a case where in the same genus, *Trifolium*, two species, *T. repens* and *T. uniflorum* (having probably the same system of self-incompatibility, i.e., one locus, multiple alleles) differ in the location of the *S* gene in the chromosome complement. It is not yet known whether the two different loci are on the same homologous chromosome or on different non-homologous chromosmes. In both cases the hybrid would be self-fertile and reciprocally cross-compatible with both parents.

This is the first case in which two normal self-incompatile species produced a self-compatible hybrid. The crossability of the hybrid with both parents, one of which (*T. repens*) is a widely used commercial pasture crop, makes it very suitable material for breeding better varieties of clover for fodder and honey. There is a possibility of tackling the problem of seed production in *T. repens* through the utilization of this hybrid in the breeding program. Backcrossing of this hybrid to a commercial variety of *T. repens* coupled with selection and induced polyploidy may have great possibilities in clover breeding. These potentialities of the hybrid are being explored by the author.

TABLE I. Reactions of the hybrid in reciprocal crosses with its parents. All the crossess would be compatible whether the *S* genes in the two species are loosely linked or completely independent

Cross (♀ × ♂) Parents with Genotype	Pollen Genotypes Incompatible	Compatible	Crossable + or Non-Crossable—
T. uni. ($T_1T_{2'0}t_{0'0}$) × Hybrid ($T_1t_0S_{2'0}$)	T_1S_2, T_1t_0	t_0S_2, t_0t_0	+
T repens ($t_0t_0S_1S_2$) × Hybrid ($T_1t_0S_{2'0}$)	T_1S_2, t_0S_2	T_1t_0, t_0t_0	+
Hybrid ($T_1t_0S_{2'0}$ × *T. uni.* ($T_1T_{2'0}t_{0'0}$)	T_1t_0	$T_{2'0}t_{0'0}$	+
Hybrid ($T_1t_0S_{2'0}$) × *T. repens* ($t_0t_0S_1S_2$)	t_0S_2	t_0S_1	+

Summary

A hyrid plant was obtained from a cross between two self-incompatible species of *Trifolium, T. uniflorum* L. × *T. repens* L. The chromosome number in *T. repens* is known to be 32 (*2n*). From root tip smears it was ascertained that *T. uniflorum* also has 32 (*2n*) chromosomes. The hybrid is vigorous and intermediate in characters between the two parental species. It is self-fertile and crosses reciprocally with both of its parents. Self-fertility of the hybrid is explained on the assumption that the *S* gene in the two species are located at different loci, either on the same homologous chromosome with considerable crossing-over, or on different non-homologous chromosomes.

Literature Cited

1. Ascherson, P. and P. Graebner. Synopsis der Mitteleuropaischen Flora. Band VI, Teil 2. 1906-1910. Englemann, Leipzig.

2. Atwood, S. S. Genetics of cross-incompatibility among self-incompatible plants of *Trifolium repens*. *Jour. Amer. Soc. Agron.* 32:955-968. 1940.

3. Brewbaker, J. L. Studies of oppositional alleles in *Trifolium nigrescens*. *Hereditas* 41:367-375. 1955.

4. ———— and W. F. Keim. A fertile interspecific hybrid in Trifolium. *Amer. Nat.* 87:323-326. 1953.

5. Guravich, D. A. Interspecific compatibility within the genus Trifolium. Doctoral thesis. University of Wisconsin. 1949.

6. Hayman, D. L. The genetic control of incompatibility in *Phalaris coerulescens* Desf. *Australian Jour. Biol. Sci.* 9:321-331. 1956.

7. Hegi, G. Illustrierte Flora von Mittel Europa. Band IV, Teil 3. 1925. Lehman, Munchen.

8. Keim, W. F. Interspecific hybridization studies in Trifolium and Lotus utilizing embryo culture techniques. Doctoral Thesis. Cornell University. 1952.

9. ———— Interspecific hybridization in Trifolium utilizing embryo culture techniques. *Agron. Jour.* 45:601-606. 1953.

10. Lewis, D. Incompatibility in flowering plants. *Biol. Revs.* 24:472-496. 1949.

11. Lundqvist, A. Studies on self-sterility in rye, *Secale cereale* L. *Hereditas* 40:278-294. 1954.

12. ———— Genetics of self-incompatibility in *Festuca pratensis*. *Hereditas* 41:518-520. 1955.

13. Pal, B. P. and Pushkarnath. Genetic nature of self- and cross-incompatibility in potatoes. *Nature* 149:246-247. 1942.

14. Pushkarnath. Studies on sterility in potato. IV. Genetics of incompatibility in *Solanum aracc-papa*. *Euphytica* 2:49-58. 1953.

15. ———— Studies on sterility in potatoes. V. Genetics of self- and cross-incompatibility in *Solanum rybinii*. *Indian Jour. Genet. & Pl. Breed.* 5:83-90. 1953.

16. Trimble, J. P. Interspecific hybridization studies in the genus Trifolium. Master's Thesis. Pennsylvania State College. 1951.

17. Wexelsen, H. Chromosome numbers and morphology in Trifolium. *Univ. Cal. Publ. Agric. Sci.* 2:355-376. 1928.

18. Williams, R. D. and R. A. Silow. Genetics of red clover (*Trifolium pratense* L.). Compatibility. I. *Jour. Genet.* 27:341-362. 1933.

19. Williams, W. Genetics of incompatibility in alsike clover, *Trifolium hybridum*. *Heredity* 5:51-73. 1951.

PRENATAL MORTALITY IN MICE BEARING THE POLYDACTYL GENE COMPLEX

LAWRENCE B. HARDY*

THE occurrence in this laboratory of polydactylous mice and several of their derived sublines has been described by Engel[3], Strong[5], and Strong and Hardy[6]. When the luxoid subline was described it was stated that the stock also appeared to carry a "semi-lethal" factor[6]. This observation was based on a smaller average litter size of offspring at birth in comparison with the litter size of young at birth of mice of the original non-polydactylous ancestral stocks. The degree of lethality was poorly expressed due perhaps to several unrecognized variables among which was the cannibalism of mothers.

It is the purpose of this paper to determine in detail the "actual litter size." The term "actual litter size" shall mean the total number of embryos produced by fertilization regardless of their survival to parturition or subsequent prenatal death and disappearance. The evidence for disappearance of embryos shall be the counting of moles.

Materials and Methods

All of the animals used in this experiment were born and raised in this laboratory. The origin of these mice has been discussed by Strong and Hardy[6]. It should be reiterated that these animals are a single independent subline characterized by several mutations that occurred in an inbred line of mice whose ancestors, the NHO, had received methylcholanthrene for many generations. They themselves, however, have not received this carcinogen since the discovery of the polydactylous condition.

The laboratory conditions have been kept fairly constant; temperature 68°-75°F, humidity 50-60 percent. Wooden boxes painted with non-lead (titanium) white paint were used. Litter of kiln dried sawdust and a little absorbent cotton for nesting material were also used. These boxes were cleaned and disinfected with Roccal at weekly intervals. "Mouse Blocks" was the staple food, with supplements of mixed grains (wheat, oats, sunflower seeds) and calf meal pellets. This regime has proven to be highly conducive to good reproduction through the many years of the Strong mouse colony.

The animals used were of two groups, experimental and control. The experimental animals were polydactylous mice from the main breeding colony, and were of the "special descent." The term "special descent" refers to those mice selected because of the presence of polydactylia on all four feet and the high number of luxoids that they threw. No selection was made in regard to their ordinal litter number.

The controls were normal toed mice of the same "special descent." The term normal means that the mice did not exhibit any of the polydactyl or luxoid characteristics, such as polydactylia, luxoid, open eyelid, mild anemia, hydrocephalous, and alopecia[6]. Normal appearing mice (somatic overlaps) may, however, have some of the complex polydactyl or luxoid syndrome, since a normal mouse of this descent has given polydactylia when outcrossed to a mouse of the C57 strain. The C57 strain has been proven to be free of polydactylia or any of its related morphological characteristics.

All animals of both groups were penmated, usually two females and two males to a pen. The colony was observed twice a week for pregnancies. When animals were found to be in late preg-

*Biological Station, Roswell Park Memorial Institute, Springville, N. Y. This experiment has been made possible, in part by a grant from the National Heart Institute of the United States Department of Health, Education and Welfare (H2666) to Dr. L. C. Strong.

nancy of about 14-15 days they were sacrificed by ether and autopsied immediately.

The uteri were removed *in toto* and placed in a petri dish containing a small amount of physiological saline solution. The uteri were stretched considerably as described by Hollander and Strong[1] so that the embryos and "moles" (partially reabsorbed embryos and placentae) could be observed and recorded. All embryos were removed and examined for morphological abnormalities, such as polydactylia, luxation, craniorachischisis, pintail, loop tail, as well as viability. The viable to dead ratio was determined for each pregnant female. Embryos were considered dead when they were being absorbed or when circulation had obviously failed.

Results

The following data are the compilation of observations of 40 pregnant mice in each group. There was no difficulty encountered in any of our observational techniques. Even the most completely absorbed embryos and placentae were observable because the placental scars and the disintegrating vasculations were still discernible.

In Group I (poly × poly) a total of 241 fetuses and moles were found. There were 171 (71.1 percent) viable found and 70 (28.9 percent) moles. In this group, 124 embryos (74 percent) were polydactylous to some degree—no separate classifications for each foot were made. Of the 127 polydactyls, 12 (11.1 percent) were also luxoid.

In Group II (normal × normal) 262 fetuses and moles were found. Two hundred and twenty-eight (87.4 percent) were viable, 34 (12.6 percent) were moles and no polydactyls or luxoids were found (see Table I).

It is noted in Table II that the average viable litter size for normal mice of the polydactylous descent mated to normal males is 5.70 mice; when the percentage of moles is added the resultant litter size is 6.44 mice. In the polydactyl × polydactyl matings, the litter size of viable embryos is 4.25; after the percentage of moles is added the size is 5.40 mice. Litter size in mice of the special descent in the original Strong colony where selection was carried out on the basis of maternal age, the average was found to be 5.57 mice for the first litters and 5.97 for the fourth litters. Penetrance of the polydactyl gene in the

TABLE I. The total data for both experimental and control groups are tabulated. The column "Total Number of Embryos" is the sum of the "Number of Viable" and "Number of Moles"

Group no.	Number viable embryos	Percentage viable embryos	Number moles	Percentage moles	Number poly	Percentage poly	Total number embryos
I	171	71.1	70	28.9	127	74.0	241
II	228	87.4	34	12.6	0	0	262

TABLE II. Average litter size of viable embryos are compared with viable plus dead embryos

Group no.	Number animals in group	Number viable embryos	Average litter size of viable young	Average litter size of entire group
I	40	171	4.25	6.25
II	40	228	5.70	6.55
C57*				8.8
NHO*				8.9

*Ancestral stocks obtained by Hollander and Strong[1].

F_{10}-F_{12} was found to be 75.27 percent of all the young in the early litter descent[7]. The present study was based only on young female mice which were having either first litters or not more than their fourth.

Discussion

The data of this experiment clearly indicate that the polydactyl gene described by Strong is inherited as a dominant. When normal mice were mated *inter se* the ensuing embryos were devoid of polydactylia.

In Table I it is noted that the experimental group of animals (poly × poly) had a very low viability of about 71.1 percent as opposed to the controls (normal × normal) that had a viability of 87.4 percent.

Group II shows that 12 percent moles are present whereas in Group I the percentage is about twice as many—27 percent. When reporting an average prenatal mortality of six inbred strains, Hollander and Strong[4] reported that it "is remarkably constant, about 15 percent." Thus the data of our normal × normal mice compared favorably with the normal mortality of other inbred strains.

Hollander and Strong[4] indicate that the average litter size for the two ancestral stocks of our polydactylous stock was about 8.8 mice. Unpublished data of Strong indicates that the range averages from about 5.57 for first litters to 5.97 for fourth litters. When the mortality percentage (moles) is added to this data we find that the resulting litter size would be 7.83 for first litters and 8.39 for fourth litters. When the average litter size of this experiment for the polydactylous × polydactylous is treated the same way we find that the litter size is 5.98. When the same treatment is given to the data of the experimental group the litter size averages 6.44 mice. This indicates that the presence of polydactylia (under maternal age selection influence) reduces the litter size considerably.

Danforth and de Aberle[2] in 1928 counted corpora lutea and found that the average count was 8.4 per female. Their data does not indicate whether this includes dead embryos. Burdick and Crump[1] using albinos of the Rockland strain reported an average litter size of living young (as determined by actual beating hearts) to be 7.8 at 10 days gestation. This may indicate that there is little mortality between the 10-14 day period. Thus our data based on 14-15 days gestation period apparently include all young that were produced by the fertilization.

Summary

The present data indicate that the presence and the expression of the polydactyl gene reduce the viability of the young *in utero*. Normal animals, however, of the polydactyl descent show a mortality equal to one-half. This is quite similar to mice of other inbred lines. When the litter size is viewed in comparison with the average litter size of two of the ancestral strains (the C57 and NHO) there appears to be a reduction in litter size whether the expression of the polydactyl gene complex is present or not. The influence of maternal age selection may also play a significant role in changing the size of the litter.

Literature Cited

1. Burdick, H. O. and Malcolm Crump. The effect of induced ovulation on pregnancy. *Endocrinology* 48: 273-276. 1951.

2. Danforth, C. H. and S. B. de Aberle. The functional interrelation of the ovaries as indicated by the distribution of foetuses. *Am. Jour. Anat.* 41: 65-74. 1928.

3. Engel, Richard. A description of polydactylism in mice. *Jour. Hered.* 47: 181-187. 1956.

4. Hollander, W. F. and L. C. Strong. Intra-uterine mortality and placental fusions in the mouse. *Jour. Exp. Zool.* 115: 131-150. 1950.

5. Strong, L. C. The origin of some inbred mice. II. Old techniques and new. *Jour. Nat. Can. Inst.* 15: 1417-1425. 1955.

6. ———— and L. B. Hardy. A new luxoid mutant in mice. *Jour. Hered.* 47: 277-284. 1956.

7. ————. Divergent penetrance of polydactylia in mice. *Proc. Assoc. Cancer Research* 2: 254. 1957.

GENE STUDIES IN TOMATO SPECIES HYBRIDS

Segregation of *Lycopersicon esculentum* Marker Genes in Backcross Progenies from Hybrids with *L. chilense*

R. K. Soost and J. W. Lesley*

THE importance of members of the subgenus *Eriopersicon*[10] in tomato breeding has been indicated by Lesley[5], Rick and Smith[12] and Doolittle[1]. They also have discussed the difficulties encountered when attempting hybridization of *Lycopersicon esculentum* with *L. peruvianum*. However, hybrids between *L. esculentum* and *L. chilense* were obtained by Lesley and Lesley[7] without the aid of embryo culture. Rick and Lamm[11] also obtained hybrids, using an accession (LA 143) which they classify as *L. chilense*. Because of the comparative ease of obtaining hybrids, the progenies reported in the present study were obtained with *L. chilense* as the representative of the subgenus *Eriopersicon*. These progenies offered an opportunity to observe the expression of marker genes contained in *L. esculentum* when introduced into the subgenus *Eriopersicon*.

Materials and Methods

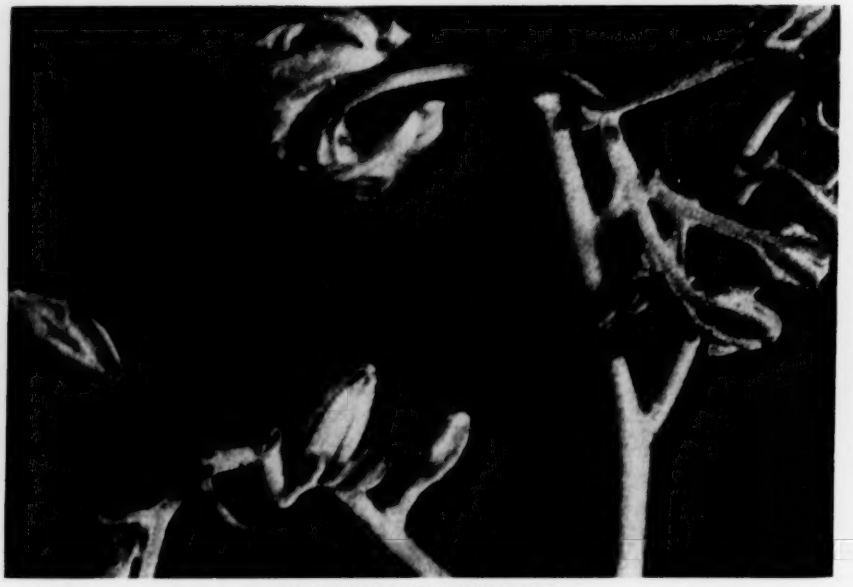
The pollen parent was a single plant from seed supplied by Dr. F. O. Holmes[4]. This seed originated from a plant established from a single seed taken from sheet 5589 of the Gray Herbarium, the specimen on that sheet having been classified as *L. chilense*. The pollen parent plant is the same one that produced the hybrids obtained earlier by Lesley and Lesley[7]. Its comparatively easy crossing with *L. esculentum*, together with its taxonomic characters, definitely indicate it to be *L. chilense* rather than a form of *L. peruvianum* var. *dentatum*.

Six F_1 plants (132-1 through 132-6) were obtained by using an *L. esculentum* plant heterozygous for *d, a, c, r, y, j,* and *f*. Each of these F_1 plants was crossed with a homozygous *c, a, j, f* tester plant. One F_1 (132-4) was also crossed with a homozygous *d, c, l, r, y* tester plant and with a homozygous *d, p, o, s, r, y* tester. Another F_1 (132-6) was crossed with a homozygous *wt, j, br* tester. All resulting progenies were scored for *d, c, a, r, y,* and *j*. One F_1 (133-1) was obtained by using an *L. esculentum* stock homozygous for *d, c,* and *l* and heterozygous for *a, r,* and *y*. This F_1 was crossed with a *d, c, l, a, r, y* tester, and the resulting progenies were scored for these genes.

Results

All the F_1 plants obtained were unfruitful and partially pollen sterile, but their pollen set seeded fruits when applied to *L. esculentum* plants. Of the six F_1 plants obtained with the seed parent heterozygous for *j*, 132-2 and 132-6 were phenotypically *j*. This, of course, indicated that the *L. chilense* parent was at least heterozygous for *j*. The *L. chilense* parent had died before the F_1 plants were examined, but a photograph of the inflorescence (Figure 11) indicates that it was also phenotypically *j*. The backcross data (Table I) demonstrate that the other four F_1 plants from this cross were heterozygous for *j*. None of the other recessive genes present in the *L. esculentum* parents were expressed phenotypically in the F_1 plants.

*Department of Horticulture, University of California Citrus Experiment Station, Riverside, California. Paper No. 977, University of California Citrus Experiment Station, Riverside.



LYCOPERSICON CHILENSE

Figure 11

This pollen parent, which is phenotypically *j*, shows lack of pedicel joints.

Segregation for *a* was not distinct even in the families where segregation is indicated (Table I). All F_1 plants showed only slight development of anthocyanin. The presence of some anthocyanin in all F_1 plants indicates that the *L. chilense* allele of a^+ allows formation of the pigment even in the presence of *a*. However, the lack of full development of anthocyanin indicates a suppression of a^+ by the *L. chilense* genotype. In scoring the progenies for *a* the segregation was most definite about 10 to 14 days after sowing. At later dates seedlings rated as showing intermediate development of anthocyanin became increasingly difficult to separate from *a* plants. If the seedlings were classified at an early date into two categories, no anthocyanin and slight to much anthocyanin, good agreement with an expected 1:1 backcross ratio is indicated. In progenies from 132-6, all seedlings rated *a* on initial scoring remained so in subsequent scorings. If a partially dominant suppressor gene *S* is postulated in *L. chilense*, then phenotypes *a* s^+ and *a S* possess no anthocyanin, phenotype a^+ *S* may show partial development of anthocyanin under some conditions, and phenotype a^+s^+ would show full development of anthocyanin. Progenies from 132-3 and 132-4 (Table I) particularly when classified soon after emerging, have an excess of plants showing development of anthocyanin. In fact all 13 plants of one family showed some development of anthocyanin at the time of classifying. This indicates that the progenies from these two F_1 plants contained no homozygous *a* plants, but that the plants scored as lacking anthocyanin were actually heterozygous for a^+ or its *chilense* allele; yet development of anthocyanin was suppressed by the *S* gene. In one family resulting from crossing 132-4 with a tester homozygous for a^+, all seedlings were classified as possessing some anthocyanin, again indicating that

suppression of a^+ is not complete. The progenies from 133-1 (Table I) scored for *a* in the field as mature plants, show more *a* plants than expected and again indicate the increasing suppression of a^+ as the plants mature.

The tester stocks used did not contain the *al*, *ay*, or *aw* genes. Therefore, the effect of these genes on anthocyanin development probably was not a factor in the behavior of these progenies even though the *L. chilense* parent may have contained one or more of them.

The flesh color of the few fruits set on the F_1 plants was ochraceous-buff-color 15'b according to Ridgeway[13]. Many plants in the progenies from the F_1 plants failed to set fruits, and segregation for r^+ among those having fruits was not sharp. All the scored progenies from the F_1 plants crossed with the stock homozygous for r^+ had plants with some visible red in the fruits although the intensity of the red was variable and some plants were without any detectable red color. The lack of visible red color in the F_1 plants and in some plants of all progenies indicates that red color cannot develop in the presence of some one gene or several genes from *L. chilense*. Although the number of plants is small the indication is that the absence or presence of visible amounts of red color is controlled by a single dominant gene allelic to r^+. One F_1 (132-4) which was crossed with the stock homozygous for r^+ and also to the one homozygous for *r*, had some red-fruited plants in all progenies (Table I) and thus demonstrated the presence of r^+ in this F_1. The progenies resulting from crossing 133-1 with a stock homozygous for *r* contained no plants with red color, demonstrating the lack of r^+ in the F_1 and the lack of r^+ or an equivalent gene in *L. chilense*.

Lesley and Lesley[6] classified populations of derivatives from *L. hirsutum* and *L. peruvianum* var. *dentatum* (*L. chilense*) for fruit color. Using seven color classes as their basis, they concluded that these species differ from *L. esculentum* by at least three genes affecting fruit color and that these genes are partially dominant. The present data do not contradict their data but indicate that lycopene is developed to a slight extent, if at all, when r^+ is absent and that there is one gene in *L. chilense* that almost fully restricts the development of lycopene even when r^+ is present. The isolation of *B* by Lincoln and Porter[8] from *L. hirsutum*, and its interactions as determined by Tomes *et al.*[16], indicate that a similar if not identical gene could be operating in *L. chilense*.

The F_1 plants had fruits with pale-yellow skin, and skin pigmentation in the backcross progenies was most variable, with only a few nonpigmented plants occurring even in some progenies from crossing the F_1 plants with stocks homozygous for *y*. Even less expected was the occurrence of nonpigmented skin on some plants in progenies from

TABLE I. Segregation of the *Lycopersicon esculentum* marker genes in backcross progenies from hybrids with *L. chilense*

F_1 * plants	Tester † stock	*c*	c^+	*j*	j^+	*d*	d^+	*l*	l^+	*r*	r^+	*a*	a^+	*y*	y^+
132–1	1	0	39	22	17	0	39	0	39	12	14	—	—	0	26
132–2	1	0	95	95	0	0	95	0	95	—	—	—	—	—	—
132–3	1	129	108	49	64	0	113	0	113	5	6	44	55	0	11
132–4	1	136	130	53	44	0	108	0	108	2	5	43	54	0	7
	2	24	24	0	23	2	46	0	23	4	4	16	33	—	—
	3	—	—	—	—	—	—	—	—	19	25	0	46		
132–5	1	0	72	36	36	0	72	0	72	—	—	—	—	—	—
136–6	1	243	191	141	0	6	241	0	119	—	—	91	91	2	15
133–1	2	101	84	0	147	82	86	82	67	146	0	49	29	13	65
	4	0	18	18	0	0	18	0	18	—	—	9	6	3	4

* The *L. esculentum* parent of plants 132-1 through 132-6 was heterozygous for *d*, *a*, *c*, *r*, *y*, *j*, *f*; the parent of 133-1 was homozygous for *d*, *c*, *l* and heterozygous for *a*, *r*, and *y*.

† Tester stocks: 1) *c*, *a*, *j*, *f*; 2) *d*, *c*, *a*, *l*, *r*, *y*; 3) *d*, *p*, *a*, *c*, *r*, *y*; 4) *ut*, *j*, *br*.

crossing F_1 plants with stocks homozygous for y^+. Therefore, *L. chilense* must possess the potential for producing skin pigment, but the development must be restricted by suppressor genes. Both Lesley and Lesley[6,7] and MacArthur and Chiasson[9] demonstrated that the skin color of F_1 plants of *L. esculentum* × *L. hirsutum* or *L. chilense* was intermediate when the *L. esculentum* parent was homozygous for y^+. MacArthur and Chiasson[9] also indicate that the F_1 was clear-skinned when the *L. esculentum* parent was homozygous for *y*. Although the constitution in relation to *y* of our F_1 plants is in doubt because the *L. esculentum* parents used in our trials were heterozygous for *y*, none of the F_1 fruits were rated as completely lacking in skin color. The abnormal segregations in the backcross progeny also indicate that genes other than y^+ are contributing to pigmentation of the fruit epidermis.

Chi-square analyses of the segregation data for *j*, *d*, and *l* indicate no significant deviations at the five percent level from an expected 1:1 ratio. Segregation for *c* approached the level of a significant difference for a 1:1 ratio in two progenies. Although it was not difficult to score for *j*, *d*, *l*, or *c*, there was a considerable amount of variability in the expression of both *d* and *c*. The occurrence of plants which were rated as *d* in progenies of 132-4 and 132-6 (Table 1) was unexpected and is without adequate explanation. Other progenies from the same F_1 plants did not contain *d* plants. These plants must have been dwarfed by action genes other than *d* or by chromosome irregularities. The presence of similar dwarfed plants in other populations derived from crosses of *L. esculentum* with *Eriopersicon* species has been reported by Rick and Smith[12]. However, their occurrence in families from backcrosses to *L. esculentum* is not to be expected.

Discussion

The behavior of the marker genes from *L. esculentum*, included in these trials, when introduced into a species of the subgenus *Eriopersicon* indicates that some *L. esculentum* genes will be manifested as they are within the species of origin. The expression of others is greatly modified either by the action of their alleles or by epistatic modifiers in the *Eriopersicon* species. The expression of *j* in the F_1 also demonstrates that some genes present in *L. esculentum* may also be present in the subgenus *Eriopersicon*. In part, at least, the similarities as well as the differences of the gene systems of *L. esculentum* and *L. chilense* are demonstrated. Therefore, in these interspecific crosses the behavior of *L. esculentum* genes when combined with *L. chilense* genes cannot be predicted from their behavior in an *L. esculentum* background. The difference in gene constitution between *L. esculentum* and *L. chilense* is paralleled by indications of general pachytene homologies of the chromosomes of *L. esculentum* with *L. hirsutum* and *L. peruvianum* but with some differences in several chromosomes[7,2].

Although MacArthur and Chiasson[9] report an approximate 1:1 segregation for the recessive genes they tested in crossing with *L. hirsutum*, Sawant[14] indicates significant differences from expected numbers in backcross and F_2 populations from crosses of two lines of *L. hirsutum* with *L. esculentum*. He found that the recovery of $sp+$ and $a+$ was less than expected but that $c+$, ms_2+, $Wo+$, and $d+$ were in excess. Sawant[14] discusses his results in relation to those obtained by Stephens[15] and Harland[3] in cotton. Because of the deficiencies of the *L. esculentum* genes that Sawant[14] observed when backcrossing to *L. esculentum*, the data do not fit the explanation suggested by Stephens[15] for cotton, where the donor parent genes are in excess. The differences in the behavior of the several genes tested by Sawant[14], and the behavior of *a*, y^+, and $r+$ in our material, suggest that deficiencies or excesses may be due to the interaction of systems of modifiers, as suggested by Harland[3] for cotton. Inheritance of a character may be simple in *L. esculentum* but polygenic in the species crosses and may account for some of the difficulty in transferring genes from the subgenus *Eriopersicon* to *L. esculentum*.

Summary

Seven F_1 plants of *Lycopersicon esculentum* × *L. chilense* were obtained from two stocks of *L. esculentum* containing a total of eight recessive marker genes. Progenies obtained by backcrossing the F_1 plants with *esculentum* stocks homozygous for these genes were analyzed for the segregation of these genes.

Segregations of the *c*, *j*, *d* and *l* genes showed no significant deviations from the expected monogenic inheritance. The $a+$ gene behaved as if altered in its behavior by a partially dominant suppressor gene inherited from

L. chilense. Expression of *r*+ and *y*+ were greatly modified. Little if any lycopene was developed unless *r*+ was present and then only in about 50 percent of the plants. The numbers of plants expressing *y* was usually deficient; however, plants classified as phenotypically *y* occurred in progenies where they were not expected.

It is suggested that the phenotypic expression of some *L. esculentum* genes may be greatly altered by genes introduced from *L. chilense* or from other species of the subgenus *Eriopersicon*.

Literature Cited

1. Doolittle, S. P. The use of wild Lycopersicon species for tomato disease control. *Phytopath.* 44: 409-414. 1954.
2. Gottschalk, W. Die Chromosomenstruktur der Solanaceen unter berücksichttigung phylogenetischer Fragestellungen. *Chromosoma* 6: 539-626. 1954.
3. Harland, S. C. The genetical conception of the species. *Biol. Rev.* 71: 83-110. 1936.
4. Holmes, F. O. The Chilean tomato, *Lycopersicon chilense*, as a possible source of disease resistance. *Phytopath.* 29: 215-216. 1939.
5. Lesley, J. W. Plant breeding methods and current problems in developing improved varieties of tomatoes. *Econ. Bot.* 2: 100-110. 1948.
6. ———— and Margaret M. Lesley. Flesh color in hybrids of tomato. *Jour. Hered.* 38: 245-251. 1947.
7. Lesley, Margaret M. and J. W. Lesley. Hybrids of the Chilean tomato. *Jour. Hered.* 34: 199-205. 1943.
8. Lincoln, R. E. and J. W. Porter. Inheritance of Beta-carotene in tomatoes. *Genetics* 35: 206-211. 1950.
9. MacArthur, J. W. and L. P. Chiasson. Cytogenetic notes on tomato species and hybrids. *Genetics* 32: 165-177. 1947.
10. Muller, C. H. A revision of the genus *Lycopersicon*. *U. S. D. A. Misc. Pub.* 382. 1940.
11. Rick, C. M. and R. Lamm. Biosystematic studies on the status of *Lycopersicon chilense*. *Amer. Jour. Bot.* 42: 663-675. 1955.
12. ———— and P. G. Smith. Novel variation in tomato species hybrids. *Amer. Nat.* 87: 359-373. 1953.
13. Ridgeway, R. Color standards and color nomenclature. 43 pp. Pub. by the author. Washington, D. C. 1912.
14. Sawant, A. C. Cytogenetics of interspecific hybrids of *Lycopersicon esculentum* Mill. × *L. hirsutum* Humb. & Bonpl. Ph.D. Thesis, Univ. Calif., Berkeley, Calif. 1954.
15. Stephens, S. G. The cytogenetics of speciation in *Gossypium*. I. Selective elimination of the donor parent genotype in interspecific backcrosses. *Genetics* 34: 627-637. 1949.
16. Tomes, M. L., F. W. Quackenbush, O. E. Nelson, Jr. and Betty North. The inheritance of carotenoid pigment systems in the tomato. *Genetics* 38: 117-127. 1953.

THE SEX CHROMOSOMES OF THE MAIDENHAIR TREE

EDWARD GENE POLLOCK*

THE chromosome number of the Maidenhair tree (*Ginkgo biloba*) is $2n = 24$. This number was first reported by Ishikawa[8], and has been verified by Herzfeld[6], Shimamura[12], Sax and Sax[11], Tanaka *et al.*[13], Newcomer[10], and Lee[9]. A haploid number of 16 was found by Illick[7]; and Chamberlain[4] reported a chromosome number of $2n = 16$. Three male and two female trees on the grounds of the University of Virginia have 24 chromosomes.

The first report of sex determination in plants, based upon a difference in the chromosomes, was made by C. E. Allen[1,2]. Subsequently, chromosomes associated with sex have been found in many dioecious plants. It was suggested by Herzfeld[6] that male and female *Ginkgo* trees might be found to differ in the morphology of their chromosomes. Eichorn[5] was the first to report the existence of satellites in the maidenhair tree, and suggested that these might be of some significance in sex determination. Sax and Sax[11] studied the chromosomes of the female gametophyte in some detail, but overlooked the satellites.

More recently Newcomer[10] and Lee[9] have described chromosomes which they consider may be sex chromosomes. Their observations, however, are not in agreement. Newcomer[10] tentatively considers that sex determination is associated with the longest pair of chromosomes which are described as dimorphic in the male. One of the members of this pair is medianly constricted, while the other is sub-medianly constricted and slightly longer. He has found that the medianly constricted chromosome of the pair occurs in approximately half the microspores and the sub-medianly constricted one in the other half.

Lee[9] found that in the ovulate tree there are four chromosomes with satellites, while in the staminate tree there are only three. The ovulate tree has a small pair of chromosomes which has satellites, while in the staminate tree only one chromosome of this pair has a satellite. It is on the basis of this single chromosomal difference that Lee infers an XY type of sex determination.

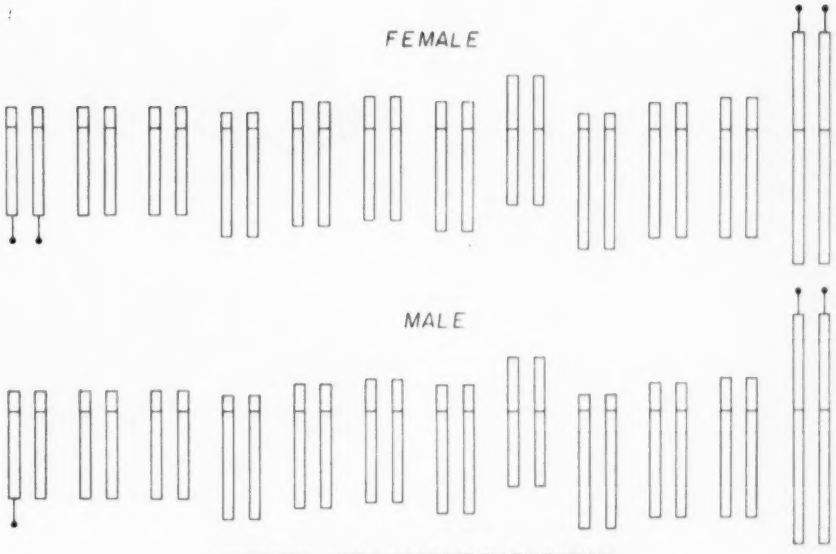
These works have led to the supposition that *G. biloba* possesses an XY type of sex determining mechanism, the nature of which is explained in terms of the female tree producing one type of gamete; i.e., possessing an X type chromosome; and the male tree producing two different types of gametes; i.e., one with an X and one with a Y chromosome.

Since Lee ascribes the absence of a satellite on one of the smaller chromosomes in the male as being significant in sex determination, and Newcomer proposes that the largest chromosome pair is associated with sex, it seems desirable to re-examine the chromosomes of the two sexes. The need for such a re-examination is evident when one notes that Newcomer's figures have recently been incorporated in a botanical textbook.†

The chromosomes of staminate and ovulate trees have been examined in considerable detail and it is now clear

*Department of Biology, University of Virginia, Charlottesville. The author wishes to express his gratitude to Dr. Ladley Husted of the Department of Biology, University of Virginia, for his invaluable criticisms and advice during the course of this study.

†*Plant Classification* by Lyman Benson. Newcomer's material is represented as an example of an heteromorphic pair of chromosomes thought to be associated with sex determination.



SOMATIC CHROMOSOME KARYOGRAM

Figure 12

Karyograms of somatic chromosomes of male and female maidenhair trees. See text for details.

that the only difference between the sexes is the presence of a satellite on the long arms of two small, subterminally constricted chromosomes in the female and the absence of one of these satellites in the male.

Materials and Methods

Young leaves were collected from one male and two female trees on the grounds of the University of Virginia in late April. Staminate cones were collected from two trees during the development of the gametophyte in May. The material was fixed in acetic-alcohol (one part glacial acetic acid and three parts of absolute ethyl alcohol) and stored at 5° C. Temporary aceto-carmine squashes were made from leaf material that had been hydrolized in equal parts of concentrated hydrochloric acid and absolute ethyl alcohol for one-half hour[14]. Microspores were stained with aceto-carmine and flattened. Observations were made through a Bausch and Lomb microscope using 10×, 15×, and 20× oculars, and a 1.8 mm oil-immersion objective. The drawings were made with the aid of a camera lucida.

Observations and Discussion

The somatic chromosomes in young leaves of *Ginkgo* are not as difficult to analyze as has been formerly maintained. Divisions are numerous if collections are made at the proper time of the year. Chromosomes at anaphase are the best for study since the constrictions are readily identified as poleward movement in progress, and the satellites are easily seen.

The diploid complement consists of 12 pairs of chromosomes. Three of these pairs are submedianly, while the remaining nine pairs are sub-terminally constricted (Figure 12). It is the first, fifth, and seventh largest pairs which have sub-median centromeres. Though Sax and Sax[11] and Lee[9] reported only two submedian chromosomes, their karyograms actually depict nine sub-terminal ones and not ten. The somatic chromosomes range in length from 5.6-12.3μ with three pairs representing the shortest group. The next longer one is comprised of three pairs all identical in total length. The third and fourth longer groups are made up of two pairs each, while

the longest and second longest groups are each represented by a single pair. The lengths of chromosome arms and total chromosome lengths are given in Table I. The values are identical for anaphase chromosomes of both male and female trees. They do not represent average lengths, but are true measurements of all the chromosomes of two cells which were especially clear in every detail.

Approximately 50 cells at metaphase or anaphase were observed in which four satellites were apparent in the female and only three satellites could be distinguished in the male. The entire complement of each of these was not analyzed, but many cells at anaphase were studied completely, thereby revealing the relative arm lengths of all the chromosome pairs (Figure 13).

In the female, satellites are located on the short arms of the longest, sub-medianly constructed pair of chromosomes, and on the long arms of one of the shortest, sub-terminally constricted pairs. In the male, satellites are located on the short arms of the longest, sub-medianly constricted pair of chromosomes, while only *one member* of the shortest sub-terminally constricted pair has a satellite (Figure 12). Thus, the gross difference is that the female has four chromosomes with satellites, while the male has three. This heteromorphism of the shortest, sub-terminally constricted chromosome pair is found in all of the cells of male plants. Satellites occur on both members of this pair in the female.

Observations were made on microspores and in all cases satellites were present. Half the microspores had two chromosomes with satellites, while the other half had one. The largest chromosome had a satellite on its shorter arm in all of the microspores, but in half of them a satellite was found also on the long arm of one of the sub-terminally constricted chromosomes. This is a confirmation of the observations of Lee[9].

Resting nuclei in the leaves of female plants have two, three, and four nucleoli, but more commonly two. There are one, two, and three nucleoli in the resting nuclei of the male, but more commonly one. This agrees with the number of chromosomes with satellites. Since the organization of the nucleolus seems usually to be the function of a specific portion of a particular chromosome, it is probable that the organization of the nucleoli of *Ginkgo* is associated with the satellite chromosomes. Lee[9] depicts nucleoli in prophase stages of haploid cells but does not attempt to identify the chromosomes associated with them.

SOMATIC CHROMOSOMES

Figure 13

A—Somatic complement in female tree of *Ginkgo* showing one pole of the chromosomes at anaphase ($2n = 24$). The constrictions and the satellites are easily seen (3550×). *B*—Somatic complement in the male tree of *Ginkgo* also at anaphase (3550×).

Dealing principally with microspores, Newcomer[10] describes and figures the haploid chromosome complement as consisting of ten sub-telocentric chromosomes, one metacentric, and the largest chromosome as being isobrachial in approximately half the microspores, and heterobrachial in the other half. He states further that satellites are found on two of the sub-terminally constricted chromosomes; on the short arm of one and on the long arm of the other. Further mention is made of the longest chromosomes carrying no satellites. These observations are contrary to the observations made on microspores in the course of this study. Satellites were found on the longest chromosomes in all cases. No satellite was found on the short arm of a sub-terminally constricted one. It is the "dimorphic"

TABLE I. Length of anaphase chromosomes in micra arranged in order of decreasing length.

Chromosome Pair	1	2	3	4	5	6	7	8	9	10	11	12
Short arm	5.0	1.7	1.4	0.8	2.8	1.4	1.7	1.4	0.8	1.1	1.1	1.1
Long arm	7.3	5.7	5.7	6.3	3.9	5.3	4.8	5.1	5.7	4.5	4.5	4.5
Total length	12.3	7.4	7.1	7.1	6.7	6.7	6.5	6.5	6.5	5.6	5.6	5.6

chromosomes which are the longest of the complement that Newcomer suggests are associated with sex determination in *Ginkgo*.

Newcomer's conclusion, however tentative, seems unwarranted. He studied the chromosomes of a single male tree. The observations of Lee[9] and those presented here agree that the only difference in male and female trees is in the heteromorphic pair of chromosomes in the male; this heteromorphism being due, not to a difference in the longest chromosomes, but to the absence of a satellite on one of the members of a sub-terminally constricted pair. In the female there is a satellite on each member of this pair of chromosomes.

Measurements taken from Newcomer's figure *2* reveal a difference of 1.1 mm between the sub-medianly constricted member of the longest pair and the medianly constricted one. He reports a 2μ difference between the members of this pair as he observed them in separate microspores. If the magnification represented for his figure *2* is correct (5400×), a 2μ difference would be represented as a 10.8 mm difference at that magnification and would be obvious at any reasonably high magnification.

Newcomer did not measure any chromosomes of the microspores other than the long ones. He found that these chromosomes were 9μ long in half the microspores and 7μ in the other half. It seems imperative to relate the measurement of the long chromosome of a given cell to the lengths of the other chromosomes of that same cell. A series of such observations on a large group of microspores would give a more reliable estimate as to the absolute difference between the long chromosomes as they are found in different cells. Since no such relationship of chromosome lengths is found in Newcomer's paper, and if his measurements were assumed to be correct, i.e., that the heterobrachial chromosome is 2μ longer than the isobrachial one, it does not necessarily follow that this is a real difference in length.

Since the observations reported here and those of Lee[9] are not in agreement with the observations of Newcomer[10], it seems probable that the tree he examined was atypical. He reports no satellites on the longest chromosomes, but reports a satellite on the shorter arm of a small, sub-terminally constricted one, an observation not encountered in the present study. To account for this situation, it is suggested that a reciprocal translocation occurred between the shorter arm of one of the longest chromosomes with a satellite and one of the shorter arms of one of the smaller, subterminally constricted chromosomes without a satellite in the normal complement. This would move the satellite from the short arm of the long chromosome to the short arm of the sub-terminally constricted one. This change may have then become homozygous. Such an occurrence would account for the positions of satellites as reported by Newcomer; i.e., none on the long chromosomes, and a satellite on the short arm of a sub-terminally constricted one. It is further suggested that an inversion or translocation involving the centromere occurred in one of the long chromosomes moving the centromere from a sub-median to a median position. Since structural change is not uncommon in plant material, the forementioned explanation seems plausible.

It would be interesting to examine the chromosomes of the material from which Newcomer's specimen is derived to see if the situation which he describes existed there. Since the material used in the present study and that used by Lee represents *27* female and eight male trees which were examined without finding anything even similar to what Newcomer has described, it appears unlikely that the situation he reports is common in *Ginkgo biloba*.

It is the heteromorphic sub-terminally constricted pair in the male which marks the only difference between the male and female complements. An examination of the chromosomes is the only morphological method now available for an identification of the sex of a *Ginkgo* tree before it reaches maturity.

Summary

The diploid number of chromosomes in male and female trees of *Ginkgo biloba* is *24*. In the ovulate trees four chromosomes of the somatic complement have satellites, while in the staminate trees there are only three. In both ovulate and staminate trees satellites are found on the shorter arms of the longest pair of chromosomes. In ovulate trees a satellite is found on each of the long arms of two of the shorter, sub-terminally constricted chromosomes. In staminate trees a satellite is found on the long arm of only one of the chromosomes of this pair. In all of the microspores a satellite is found on the short arm of the longest chromosome. In approximately one-half of the microspores a satellite also occurs on the long arm of one of the short, subterminally constricted chromosomes. It is inferred that the heteromorphic chromosomes are sex chromosomes.

Literature Cited

1. Allen, C. E. A chromosome difference as correlated with sex differences in *Sphaerocarpos*. *Science* 46: 466-467. 1917.

2. ———. The basis of sex inheritance in *Sphaerocarpos*. *Proc. Amer. Phil. Soc.* 58: 284-316. 1919.

3. Benson, L. Plant Classification. D. C. Heath and Co., Boston. 1957.

4. Chamberlain, C. J. Gymnosperms. Structure and Evolution. Univ. Chicago Press. 1935.

5. Eichhorn, A. Sur la mitose somatique et le satellite du *Ginkgo biloba*. *Compt. Rend. Soc. Biol.* Paris 99: 1787-1788. 1928.

6. Herzfeld, S. Ueber die Kernteilung im

Proembryo von *Ginkgo biloba*. *Jahr. Wiss. Bot.* 69: 264-294. 1928.

7. ILLICK, J. T. Chromosomes of *Ginkgo biloba* during early development stages of the male gametophyte. *Anat. Rec.* 81: 78-79. 1941.

8. ISHIKAWA, M. Ueber die Zahl der Chromosomen von *Ginkgo biloba* L. *Bot. Mag.* (Tokyo) 24: 225-226. 1910.

9. LEE, C. L. Sex chromosomes in *Ginkgo biloba*. *Amer. Jour. Bot.* 41: 545-550. 1954.

10. NEWCOMER, E. H. The karyotype and possible sex chromosomes of *Ginkgo biloba*. *Amer. Jour. Bot.* 41: 542-545. 1954.

11. SAX, K., and H. J. SAX. Chromosome number and morphology in the conifers. *Jour. Arnold Arboretum* 14: 356-375. 1933.

12. SHIMAMURA, T. On the formation of the proembryo of *Ginkgo biloba* L. *Bot. Mag.* (Tokyo) 42: 71-76. 1928.

13. TANAKA, N., N. TAKEMASA and Y. SINOTO. Karotype analysis in Gymnospermae. I. Karyotype and chromosome bridge in the young leaf meristem of *Ginkgo biloba* L. *Cytologia* 17: 112-123. 1952.

14. WARMKE, H. E. A permanent root-tip smear method. *Stain Tech.* 10: 101-103. 1935.

◆

A NEW GENETIC FLOWER TYPE OF THE MUSCADINE GRAPE

N. H. LOOMIS AND C. F. WILLIAMS*

PERFECT-FLOWERED muscadine grapes were first reported in the early 1900's, by Reimer and Detjen[8], Husmann and Dearing[4] and Dearing[2]. Reimer and Detjen's vine was named "Hope"[3]. Husmann and Dearing's two original vines were designated as H^1 and $H^{2.1}$ and Dearing's third hermaphrodite as $H^{3.3}$. There is no further reference to H^3 in the literature.

Inheritance data on the perfect-flowered character were presented by Dtejen[3], Dearing[1,2], Loomis[5], and Loomis *et al.*[6] The genetic information is meager, but the following facts seem to be established: (1) Pistillates × hermaphrodites† give a 1:1 ratio of the parental types; (2) self-pollinating hermaphrodites from H^1 gives a 3:1 ratio of hermaphrodites to pistillates, and self-pollinating hermaphrodites from H^2 give approximately a 9:4:3 ratio of hermaphrodites, pistillates, and staminates; (3) crosses of hermaphrodites with normal wild staminates produce only pistillate and staminate seedlings with a considerable excess of staminates. These populations were small.

Old unpublished records at Raleigh, N. C., by Reimer and Detjen, give some data on selfed hermaphrodites of the Hope vine. The records are not complete enough to indicate the inheritance of flower type, but all populations contained staminate plants; so the Hope vine must be similar genetically to H^2. The Hope vine apparently originated as a mutation of a staminate type plant[3]. There is nothing in the literature to indicate the flower type which mutated to give the hermaphrodites H^1, H^2, and H^3.

This paper reports the discovery of a perfect-flowered vine, No. 36-2A, which appears to be homozygous for the perfect-flowered character. Progeny records from some complete populations are available (Table I). All the selections listed in Table I are indirectly from the H^2 hermaphrodite.

(*Continued on page* 304)

TABLE I. Segregation of progenies of muscadine selection 36-2A*

Progeny	Plants (number) Total	Hermaphrodite	Pistillate	Staminate
Selfed 36-2A	35	35	0	0
18-6C† × 36-2A	6	6	0	0
46-2B† × 36-2A	20	20	0	0
36-2A × 46-15A‡	1	0	0	1

*Hermaphrodite seedling originated by selfing the hermaphroditic variety, Dearing.
†Pistillate seedling originated by selfing the hermaphroditic selection B-6 19.
‡Staminate seedling originated by selfing the hermaphroditic selection B-6 19, a sibling of Topsail.

*Crops Research Division, ARS, U. S. Department of Agriculture, Meridian, Mississippi, and North Carolina Agricultural Experiment Station, Raleigh, North Carolina, respectively.
†The terms hermaphrodite and perfect-flowered are used interchangeably.

HYBRIDIZATION OF TRIPSACUM AND ZEA

L. I. Farquharson*

THE crossing of corn with related grasses has intrigued botanists for a number of years and for several reasons. There is, first of all, the possibility of introducing into the corn plant genetic material which might be of practical value in the improvement of maize. Secondly, it is supposed that the degree of ease in accomplishing the cross would indicate the closeness of the relationship between corn and its nearest relatives and hence relate to the problem of the origin of corn. Lastly, it is of theoretical interest that species of separate genera can be crossed, and perhaps this should lead to a reconsideration of the taxonomic position of *Zea*, *Tripsacum* and *Euchlaena*.

Slightly more than a quarter century ago, Mangelsdorf and Reeves[4] reported the hybridization of corn and tripsacum using corn as the maternal parent. The cross was not an easy one to achieve. It necessitated a shortening of the corn silks to a length nearly that of tripsacum styles and the pollination of a large number of silks. Many of the seeds aborted, but it was estimated that 4.54 seeds matured per 10,000 silks pollinated. Artificial culture techniques were utilized in germinating many of the hybrid seeds. This intergeneric cross was successful, however, only when corn was used as the maternal parent. Numerous crosses with tripsacum as the female parent were attempted, but only shriveled caryopses were obtained, and it was not determined whether these were a result of nucellar stimulation or were caused by the initiation of hybrid embryos or endosperm[4,5].

Since the author had growing at Indiana University a large number of plants of *Tripsacum dactyloides* from widely scattered localities and also had available, through the courtesy of Dr. Paul Weatherwax, a wide range of types of corn, the attempt was made to effect the cross using tripsacum as the maternal parent. The results include one hybrid now in bloom, several putative hybrids and numerous seeds, thus indicating that the cross in this direction is by no means an impossible one.

Materials and Methods

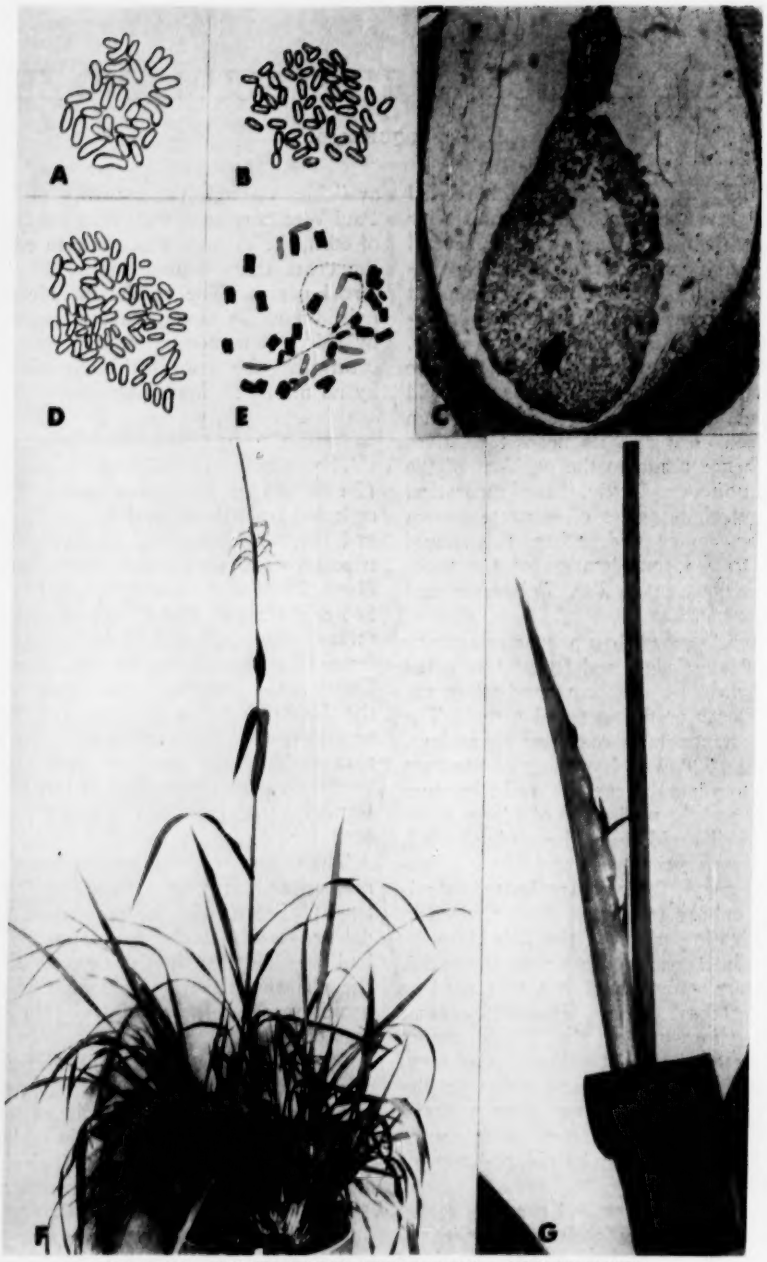
The plants of diploid tripsacum ($2n = 36$) used in these crosses were collected from three localities in Kansas and three in Missouri. The polyploid tripsacum was obtained in southern Indiana. Most of this was triploid ($2n = 54$) or tetraploid ($2n = 72$). The corn utilized for pollen included varieties from Huancayo, Puno and Urubamba, Peru; some Mandan Indian types from the Dakotas; Hopi, Navajo and Zuñi varieties from the southwest; a Tuscarora variety from western New York; tetraploid corn obtained from Dr. L. F. Randolph; and Golden Bantam sweet corn.

The styles were pollinated when fully mature and at nearly their maximum length. Frequently, the pollination was delayed until all the tripsacum pollen had been shed for the day thus reducing the chances of stray tripsacum pollen grains reaching the styles while the bag was open.

Because of possible difficulty in bringing some of the hybrid embryos to maturity, embryo culture techniques were used to a certain extent. The culture medium used was basically that devised by Randolph and Cox[6]. Some seeds matured and subsequently germinated, however, without artificial culture.

In order to obtain chromosome counts, root tips and anthers were fixed

*Franklin College, Franklin, Indiana. The author wishes to express her appreciation to Indiana University for making research facilities available for this study.



DIPLOID AND TRIPLOID TRIPSACUM-CORN HYBRIDS

Figure 14

A–C—Diploid tripsacum × sweet corn hybrid. *A*—Chromosomes from cell of embryo, $2n = 28$; *B*—Chromosomes from cell of endosperm, $3n = 46$; *C*—Longitudinal section of caryopsis six days after pollination. *D–G*—Triploid tripsacum × Puno corn hybrid. *D*—Chromosomes from root tip cell, $2n =$ ca.64; *E*—Diakinesis in microsporocyte; *F*—Mature plant; *G*—Lateral inflorescence surrounded by leaf sheath.

in 3:1 absolute alcohol-acetic acid and were smeared in iron aceto-carmine.

Results

Pollen from all the varieties of corn used was effective in at least initiating enlargement of the ovary in both diploid and polyploid tripsacum. Nucellar stimulation alone resulted in some grains attaining a length of 3 mm in 13 days compared with 4.5 mm for a normal tripsacum seed of the same age. These enlarged ovaries had a translucent, watery appearance.

Crosses with Diploid Tripsacum. In the crosses attempted between the diploid races of tripsacum and corn, approximately 24 percent of the ovaries showed significant enlargement. Crosses utilizing pollen from commercial sweet corn, one Navajo, one Mandan and all three Peruvian varieties resulted in the establishment of embryos and endosperm. Chromosome counts of 28 in cells of the embryo and 46 in endosperm cells confirmed the assumption of hybridity (Figures 14*A* and *B*). The endosperm was frequently lobed and did not fill the caryopsis (Figure 14*C*). Sometimes the embryo was lacking. The caryopsis at maturity was often almost as long as that of a normal tripsacum seed, although when it dried some wrinkling and shrinkage frequently occurred. Of the 25 seeds obtained from these crosses with diploid tripsacum, 22 were the result of pollinating tripsacum with Peruvian corn. The Puno type, from the shores of Lake Titicaca, seemed to be especially effective. Only three of these seeds germinated, and the plants produced have not yet bloomed.

Crosses with Polyploid Tripsacum. The polyploid races of *T. dactyloides* present a more complex situation because of the high degree of facultative apomixis which exists in them. Embryos are readily initiated without the occurrence of fertilization or even pollination, but pollination is necessary for endosperm development[2,3]. There is considerable variation in chromosome number in these plants. Somatic chromosome numbers of 36, 45, 54, 72, 90 and 108 have been found, although 72 chromosome plants are the most commonly encountered[1].

When the polyploid tripsacum was pollinated with corn, two to seven ovaries out of an inflorescence averaging 14 spikelets showed significant enlargement, but only one in 20 of the pollinated spikelets developed a good seed. Corn pollen grains germinated readily and although many of the tubes burst in the styles (Figure 15*B*) many did not (Figure 15*C*) and a few were seen to have reached the micropylar region. Pollen tubes reached the base of the style within four hours after pollination. If the styles were removed four hours after pollination, however, embryos and endosperm were not initiated. Removing the styles six hours after applying corn pollen did not interfere with the development of embryos and endosperm. This timing is about the same as when tripsacum pollen is utilized[2]. Endosperm and embryos were initiated when the pollen parent was any one of the three Peruvian varieties, or Hopi, Zuñi, Tuscarora or sweet corn. Thirty-eight seeds were obtained and although some of the embryos were undoubtedly apomictic, the endosperm would not have developed without the stimulation of the corn pollen. The large number of chromosomes present in the endosperm made it impossible to obtain an accurate chromosome count from sectioned material.

At least one seedling was secured from each category of crosses when polyploid tripsacum was pollinated with Puno, Urubamba, Hopi, Zuñi and sweet corn. Those seedlings which had Urubamba and Hopi corn as pollen parents were abnormal and did not survive more than a few weeks. One appeared to have root and shoot orientation reversed, and the other had a curled coleoptile. It is likely that these were hybrids. Several others which germinated were discarded as being apomictic tripsacum plants. Of the two seedlings which were kept, Zuñi corn was the pollen parent of one and the Puno, Peru race the male parent of the other. Studies of meiosis indicated that the tripsacum parent of these two plants is a triploid. The triploid tripsacum × Puno corn plant bloomed this year and gave evidence of being a true hybrid (Figures 14*F,G* and 15*A*).

The hybrid plant resembles the maternal parent tripsacum far more than it resembles its corn parent. This is to be expected since tripsacum contributed the larger number of chromosomes, and it is similar to the findings of Mangelsdorf and Reeves[4] with the corn × tripsacum plants. One outstanding feature distinguishing the hybrid is the length of the style (5.5 cm. as compared with 2.5 cm. for tripsacum, measured from the point of emergence between the outer glume and rachis). There is a tendency for the two branches of the style to be fused a greater distance than in tripsacum (as much as 4 cm. in the hybrid vs. 1-2 mm. in tripsacum) thus showing corn influence, but the branches separate very readily. There is a slight compression of the rachis which tends to set the spikelets out a bit more prominently than is the case with most tripsacum plants. The lateral inflorescences tend to remain enclosed by the leaf sheaths even after the styles begin to emerge, and in this way give the appearance of small ears (Figure 14*G*). It is not unusual for the inflorescences to lack staminate spikelets. While this may be a result of corn influence, there is also the possibility that it is due to greenhouse conditions during the winter months. The leaves of the hybrid are conspicuously marked by reddish-brown patches

TRIPSACUM-CORN HYBRIDS

Figure 15

A—Terminal inflorescences. Left, tripsacum; right, tripsacum × Puno corn hybrid. *B*—Puno corn pollen tubes bursting in styles of triploid tripsacum; *C*—Puno corn pollen tubes growing normally in styles of triploid tripsacum, three hours after pollination.

which appear rust-like at first glance. It was this latter characteristic that made this plant stand out among the pure tripsacum plants when it was young and may have been all that saved it from being discarded as an apomict. The mottling is present in the tripsacum parent, but far less obviously, and it has not been fully investigated.

Cells of the root tips of the tripsacum × Puno corn hybrid showed chromosome counts in the low 60's—63 or 64 chromosomes having been counted in several cells (Figure 14*D*). At first this would seem unlikely since the tripsacum plant is a triploid with a somatic chromosome number of 54. It is not unusual, however, for polyploid tripsacum plants to produce gametes possessing the unreduced number of chromosomes. Therefore, it is assumed that the egg cell in this case carried approximately 54 chromosomes and this, when fused with the corn sperm with 10 chromosomes, produced the approximate 64-chromosome hybrid.

Meiosis has also been studied and it adds evidence in support of the chromosome number being close to 64 (Figure 14*E*). Univalents are common and it is relatively easy to distinguish 10 that are longer than those of tripsacum and presumably are corn chromosomes. In addition, there is a somewhat variable number of shorter univalents (frequently eight) and an occasional multivalent, and the rest (21-22) are bivalents. The pollen produced is inviable, and so far, no viable seeds have been set when the plant has been exposed to pollen of *T. dactyloides* and *T. floridanum*. Recently, however, two spikelets set seed following the application of sweet corn pollen to two inflorescences of the hybrid. The seeds are somewhat shriveled and it is doubtful that they will germinate.

Discussion

Perhaps the chief significance of this work lies in the fact that it has been shown that the cross between tripsacum and corn is not a difficult one to make, and requires no special technique. In fact, it seems highly probable that this cross has occurred occasionally in nature. The hybrid plants might go long unnoticed because of the close resemblance to tripsacum. Some of the sterility and meiotic irregularities which are frequently encountered in tripsacum may be the result of admixture with corn. Since the hybrid is strongly perennial, tripsacum × corn plants could persist indefinitely despite sterility.

If it can be shown conclusively that certain of the Indian varieties of corn cross more readily than others with tripsacum, one more line of evidence of relationship between the two genera would be apparent. It does seem likely that the reason previous workers did not succeed in making this cross, despite repeated efforts, was that they were utilizing commercial varieties of corn which are farther removed from the ancestral type. From the crosses made in this study, it would appear that the Puno corn, which grows along the shore of Lake Titicaca at an elevation of approximately 13,000 feet, is especially promising. Before any conclusions are drawn from this pertaining to the center of origin of corn, however, an attempt should be made to determine the ease of crossing tripsacum with corn from the region of southern Mexico and Guatemala, an area thought to be one likely center of early culture of primitive corn[7].

Summary

Tripsacum and corn have been successfully crossed, using tripsacum as the maternal parent. No special techniques are necessary, although embryo culture undoubtedly results in a higher percentage of embryos brought to maturity. A number of varieties of corn were utilized. Those grown by primitive races of Indians in Peru and in the southwestern U. S. were most successful. A living hybrid (triploid tripsacum × corn from Puno, Peru) is described. It is suggested that this cross between tripsacum and corn may occur occasionally in nature.

Literature Cited

1. Farquharson, L. I. Natural selection of tetraploids in a mixed colony of *Tripsacum dactyloides*. *Proc. Indiana Acad. Sci.* 63: 80-82. 1954.

2. ————. Apomixis, polyembryony and related problems in *Tripsacum*. (Thesis). Univ. Microfilms. Ann Arbor, Mich. 1954.

3. ————. Apomixis and polyembryony in *Tripsacum dactyloides*. *Amer. Jour. Bot.* 42: 737-743. 1955.

4. Mangelsdorf, P. C. and R. G. Reeves. Hybridization of maize, *Tripsacum* and *Euchlaena*. *Jour. Hered.* 22: 329-343. 1931.

5. ———— and ————. The origin of Indian corn and its relatives. Bulletin 574. *Tex. Agric. Exp. Sta.* 1939.

6. Randolph, L. F. and L. G. Cox. Factors influencing the germination of *Iris* seed and the relation of inhibiting substances to embryo dormancy. *Proc. Am. Soc. Hort. Sci.* 43: 284-300. 1943.

7. Weatherwax, P. Indian Corn in Old America. Macmillan Co., New York. 1954.

LINKAGE STUDIES IN A NEW BLACK-EYED WHITE MUTATION

In the House Mouse (Not *W*)

PHILIP C. KREITNER*

THE mutation involved in the experiment described below (discovered by Dr. L. C. Strong) is a black-eyed white mouse which arose as a spontaneous mutation in a mouse of the C3H inbred strain[6,7]. The mutant strain was given the designation C3HB. The B represents the second spontaneous mutation which had occurred in mice of the C3H strain during a period of 35 years. The mice in this new mutant strain appeared to be perfectly normal with respect to viability, reproduction and size, contrary to the findings of other researchers who have experimented with various types of "black-eyed whites" or "all-whites." The C3HB mutation was the first "all-white" type of mutation in the C3H strain. Most previous work with "black-eyed white" occurrences was done in association with piebald (*s*) or yellow A^y.

In the spring of 1955, this author began an experiment to determine the location on a chromosome of the mutant gene by the use of outcrosses.

The purpose of this paper is to summarize and to analyze the results of these outcrosses and several other crosses involving the mutant strain; also, to compare the C3HB "black-eyed white" mutant with the black-eyed whites or phenotypes described by earlier researchers such as Little[4,5] and Dunn[1].

Grüneberg[3] concerns himself mainly with a description of the *m*(*W*) complex, the (*s*) piebald gene, the "*k*" genes, and their role in creating genotypes, of which the genotype causing the "black-eyed white" condition is but one. In a later section of Grüneberg, "black-eyed white" is also mentioned in connection with "macrocytic anemia." However, we shall concern ourselves just with the description of the modifying genes, since it also contains a sufficient explanation of the role that anemia plays in "dominant spottings." Grüneberg states, "The dominance of gene *W* depends on the presence of a specific set of modifiers, which, by themselves, have little or no effect on spotting. In the presence of all these modifying genes, *W* behaves as a complete dominant; if accompanied by only some of them, as a semi-dominant, and in their absence as a recessive"[3].

Gene *W* produces lethal anemics in the homozygous condition whether the modifiers are present or not; in the heterozygous condition the presence of the modifiers increases the amount of white spotting of the offspring. If the modifiers are all present, the animals are black-eyed whites. The degree of spotting or variegation in the *W*/+ heterozygotes is dependent on the number of genes of the *m*(*W*) complex modifying factors which are present.

The gene (*s*) piebald spotting is another gene which affects the degree of spotting caused by gene *W*. Gene *s* in the heterozygous condition, (*W*/+ ;/+*s*) produces slightly more white on mice, than when the allele of *s*/*s*, *W*/+ ; +/+, is present. Furthermore when *s* is homozygous; (*W*/+ ;*s*/*s*), it produces the usually all-white, "black-eyed whites."

Black-eyed whites of the constitution (*W*/+ ;*s*/*s*), when mated *inter se*, produce lethal anemics, "black-eyed whites" and piebalds, in the ratio of 1:2:1. Le-

*Griffith Institute, Springville, New York. This study received honorable mention from the 1957 Westinghouse Talent Search Committee. The author gratefully acknowledges the guidance and sponsorship of Dr. Leonell C. Strong and the assistance of Mr. Lawrence W. Hardy, Biological Station, Roswell Park Memorial Insitute, Springville, New York.

thal anemics which survive long enough to show hair color are black-eyed whites. Black-eyed whites of this strain are sometimes pigmented about the ears or on the haunches.

Little [4,5] agreed with Dunn[1,2] in the observation that "black-eyed white," potentially yellow, has considerably more dorsal pigmentation than does "black-eyed white," potentially non-yellow (black). According to Little this is due either to a modifying factor linked with yellow, or to an interaction between the *Y* and *H* factors, "of a purely somatic nature."

Earlier, Little had obtained two pairs of "black-eyed white" mice from Castle, which he mated *inter se;* he also mated piebald *inter se*. Considering any animal with 5 percent or less of dorsal pigmentation, as a black-eyed white, the first cross produced 44 black-eyed whites and 31 varied piebalds, the second cross produced nearly equal numbers of black-eyed white and piebald, the third cross produced only piebalds. By comparing these results with results expected by ordinary Mendelian segregation, Little concluded that the black-eyed white condition was not viable in the homozygous condition.

In another experiment with the black-eyed white condition, Little crossed a "blaze" race, resembling a "self" race, with the piebald strain and with black-eyed whites. From the results of these crosses, in which black-eyed white animals appeared where they would not have occurred in the event of independent assortment, Little concluded that "black-eyed white spotting in mice appears to be due to a factor independent of, and supplementary to, the factor for piebald spotting." He also speculated on the possibility of the spotting condition in mice being dependent upon "more than one pair of clear-cut Mendelizing factors, and contributing to variation in spotted races"[5].

Dunn[1,2] in his experiment with piebald types of the "Dutch-marked" variety, selected animals with a maximum of white spotting, and bred them selectively, continuously inbreeding the offspring with the highest percentage of white. After three generations of selective breeding, "all-white" animals began to appear. After five generations, the mice with no black spotting whatever, the "all-whites," were mated to start an inbred stock. This inbreeding produced about 99 percent "all-whites" through 23 generations, all the while retaining complete dark eye pigmentation.

By further experimentation, Dunn came to the conclusion that the "all-white" piebalds contained not only the spotting gene (*s*), but at least three other spotting genes working independently of *s*, since they produced spotting when *s* was absent[1].

Dunn later crossed yellow piebalds (*A^{y}ss*), with black piebalds (*aass*) which resulted in equal numbers of black and yellow piebalds. However, planimetric measurements showed a significantly lesser degree of white spotting in the yellows than in the blacks. From this observation, he drew the conclusion that there may be an interaction between the *A^{y}* gene and the *m(H')* modifiers of variegated spotting[2].

The C3HB Mutant

The original black-eyed white mutant appeared in a mouse of the 89th inbred generation of the C3H strain. This mouse was a male born in a litter with two normal sisters. The mutant's dam had previously had three normal litters. Out of the 20 young she produced, 19 were normal black-eyed as expected. The original mutant was born on June 6, 1954. He was mated to his sister and the resulting black-eyed stock obtained from this mating was inbred for several generations, with no aberrations. The black-eyed white mutant bred true as a normal recessive. The mouse obtained in the original mutation, and all of the inbred C3HB stock have been pure-white animals with no trace of pigmented hair but having intense black eyes.

As previously stated, the black-eyed whites of the strain C3HB have been

normal in all respects including viability, size, prolificity and litter size. Eight adult C3HB's—five males and three females—were weighed and measured and found to have an average weight of 37 grams, an average body length of 3⅞ inches and an average tail length of 3⅝ inches. A factor which should be considered with relation to the average weight, is that all the females were pregnant at the time of weighing.

Materials and Methods

Besides the original C3H stock and its mutant strain C3HB, there were three other inbred strains concerned with the present experiment of possible linkage relations. The Strong A strain (albino), (*ppaabbccddss*)[8], the 2 prunt strain (*ppaabbccddss*)[9], and the I strain (*ppaabbccddss*)[9], all were used for outcrosses.

All of the mice used in the experiment were taken from strains housed at the Springville station. They were kept in wooden boxes, with sawdust bedding and cotton nesting material, in a building with a temperature range of 70-80° F. They were fed Nurishmix pellets and water *ad libitum;* also, mixed grain, to which cod-liver oil had been added, and milk-soaked bread were fed periodically.

The mutant strain C3HB was crossed with the Strong A strain in the first outcross, with the 2 prunt strain in the second outcross and with the I strain for the third outcross. The genes under observation for classification purposes were in the first outcross, *a* (nonagouti), *b* (brown), *c* (albino), *M* (mutant); in the second outcross, *a, b, C, M, p* (pink eye); and in the third outcross, *a, b, p, s* (piebald spotting), *d* (dilution).

A fourth cross made in conjunction with the three principal outcrosses was that of the C3HB × 2 prunt extracted pink-eyed whites. Mice of special color combinations were mated to mice of the Strong A strain in order to ascertain that a pink-eye mutant could not be separated phenotypically from an albino.

Occurrence of linkage in each outcross was tested by comparing the ratio of the totals in each classification of offspring to the ratio expected in the case of simple Mendelian segregation. The closeness of fit of the observed ratio to the expected ratio was determined by use of the chi-square test.

Results

A total of 355 mice was obtained in the first outcross, (C3HB × A). The resultant ratio in the phenotypic classes, in the F_2, closely conformed with the ratio expected as a result of independent assortment between *a, b, c,* and *m.* The excellent chi-square fit of 0.86 (above .05 being statistically insignificant) practically excluded the possibility of linkage of the mutant gene to any other of the genes under consideration in the outcross.

A total of 346 mice was obtained in the second outcross (C3HB × 2 prunt). Although there was greater deviation from the expected ratio in the second outcross than in the first, it being mostly due to a deficiency in the pink-eyed colored class, the chi-square still showed a statistically insignificant deviation from an expected ratio due to free assortment, with a value of 0.139. Here, the genes involved were *a, b, p,* and ***m.***

Other significant occurrences were the appearance, in the presence of *pp* and *mm*, of a "pink-eyed" condition, indistinguishable from the Strong A strain albino; and a "ruby-eyed" condition which appeared to be intermediate in shade between "pink-eye" and "black-eye." The ratio of "black-eyed whites" to "ruby-eyed whites" to "pink-eyed whites" was 57:20:24, closely approximating the ratio of 3:1:1.

In the "black-eyed white" or "black-eye"—"ruby-eye" classification, out of a total of 57 "black-eyed" mice there appeared five mice, each with a very small black spot, varying in size from a few hairs to about 2 cm. in diameter, on the dorsum. The grayish tone of the spots was produced by variegation, and not

TABLE I. Results of the first outcross

	Bl.Ag.	Bl.N.Ag.	Br.Ag.	Br.N.Ag.	P.-Eye-White	Bl.-Eye-White
Obs.	114	42	31	12	64	92
Exp.	112.59	37.53	37.53	12.51	66.72	88.96
Total Observed	355		$x^9 = .86$			

by incomplete pigmentation of the individual hairs.

All of the offspring obtained in the third outcross, (C3HB × I) were black agouti, despite the five recessive genes from the I stock. This indicates the probable presence of the five homozygous dominant alleles in the C3HB strain; the new black-eyed white mutant could not be allelic with any of the I strain genes, (*a, b, p, s*) (piebald spotting) and *d* (dilute).

Likewise, the progeny of the C3HB × 2 prunt extracted pink-eyed-white to Strong A cross were all black agouti.

In every outcross so far, the original C3H strain mice have possessed only dominant genes *A, B,* and *C;* all the mice of the F_1 generations were wild type or black agouti, thus indicating that the new mutation is completely recessive in inheritance.

Discussion

Considering only the genes under observation in the first outcross, the previously established constitution of the Strong A strain is (*aa, bb, cc, pp, MM*). The ratio of phenotypes in the offspring indicated that free assortment had occurred between the Strong A genes and their alleles which have been proven to comprise the C3HB strain constitution; (*AA, BB, CC, mm*).

One F_2 black-eyed white mouse was found to have a small, black, dorsal spot. However, as it was the only instance of black spotting in 92 black-eyed whites, it may be considered as the result of non-genetic factors. The original mutation in the C3H strain occasionally shows a small amount of pigmented hairs on the dorsum.

In the second outcross, if the spotting was due to genetic factors, this fact must be taken into consideration; the spotting appeared only in the "unpigmented" class which contained the homozygous allele for pink-eye (*PP, mm*). This fact supports the possible conclusion that gene *p* may interact or at least affect the complete dominance of the mutant gene; that is, *p* could be considered a modifying factor to the new mutation.

By considering both the conclusion that genes *m* and *p* interact and the fact that black spotting has arisen only in "black-eyed white" mice, the following constitutions are theorized:

"black-eye"—*PPmm*
"ruby-eye"—*Ppmm*
"pink-eye"—*ppmm* (which cannot be distinguished phenotypically from a common albino of the constitution (*ppcc*) or (*PPcc*).

The production of all black agouti progeny in the third outcross, points to the obvious conclusion that the C3HB strain contains all the homozygous dominant alleles of the five recessive genes: *a, b, p, d* (dilution), and *s* (piebald spotting).

This conclusion is further supported by the results of the C3HB extracted pink-eyed white to Strong A cross: 100 percent black agouti offspring. The Strong A strain has, in a previous experiment at Springville, been proven to be homozygous for *b* (brown), *a* (non-agouti), and *c* (albino); consequently, their dominant alleles *B* (black), *A* (agouti), *C* (color), which appeared exclusively in the progeny of the cross, must have been derived from the C3HB × 2 prunt, F_1's, who, in their turn, had derived them from the C3HB parents.

Summary

A new recessive mutation in the house mouse eliminates practically all the pigment in the pelage. The color of the eyes, however, is not affected. Occasionally a few pigmented hairs may be present on the dorsum of the mouse. The new mutation in the presence of *pp* produced a non-pigmented mouse which could not be distinguished phenotypically from an albino of the constitution *ppcc* or *PPcc*. An outcross to mice of the I stock (*aabbppssdd*) gave only wild type in the F_1 thus indicating that the new black-eyed white mutant is not allellic to any of these well known genes. Other crosses gave evidence of free assortment when genes *a, b, c,* and *p* were tested.

The new black-eyed white mutant is an extreme example of loss of pigmentation in the pelage thus similating the effect of *W/W* in the presence of its modifiers. The new mutant, however, is fully viable, recessive in inheritance and is not identical with *W* or *Wy*.

Literature Cited

1. Dunn, L. C. and D. R. Charles Studies on spotting patterns. I. Analysis of quantitative variations in the pied spotting of the house mouse. *Genetics 22*: 14-42. 1937.

2. ————., E. C. MacDowell and G. A. Lebedeff. Studies on spotting patterns. III. Interaction between genes affecting white spotting and those affecting color in the house mouse. *Genetics 22*: 307-318. 1937.

3. Grüneberg, H. The Genetics of the

TABLE II. Results of the second outcross

	Bl.Ag.	Bl.N.Ag.	Br.Ag.	Br.N.Ag.	P.-Eye Colored	P.-Eye White	Bl.-Eye White (Ruby)	Bl.-Eye White (Black)
							20	57
Obs.	113	42	30	13	47	24	77	
Exp.	109.1	36.5	36.5	12.2	64.8	21.6	64.8	
Total Observed	346			$x^2 = .139$				

Mouse. Martinus Nijhoff, The Hague. 1952.

4. Little, C. C. The relation of yellow coat color and black-eyed spotting of mice in inheritance. *Genetics* 2: 433-444. 1917.

5. ————. The inheritance of black-eyed white spotting in mice. *Amer. Nat.* 49: 727-740. 1915.

6. Strong, L. C. The establishment of the C3H inbred strain of mice for the study of spontaneous carcinoma of the mammary gland. *Genetics* 20: 586-591. 1935.

7. ————. The genetic appearance of spontaneous carcinoma of the mammary gland in the C3H mice. *Amer. Jour. Cancer* 25: 599-606. 1935.

8. ————. The establishment of "A" strain of inbred mice. *Jour. Hered.* 27: 21-24. 1936.

9. ————. The origin of some inbred mice. I. *Cancer Res.* 2: 531-539. 1942. II. Old Techniques and New. *Jour. Nat. Can. Inst.* 15: 1417-1425. 1955.

◆

A New Genetic Flower Type of the Muscadine Grape

(*Continued from page 294*)

The one staminate vine is hard to explain. However, in all crosses involving staminate plants, the staminate factor has shown complete dominance over the hermaphroditic factor. Records are available on three different types of crosses involving hermaphrodites from H^2: (1) hermaphrodites × normal (wild-type) staminates; (2) the backcross — hermaphrodites × staminates (from the cross hermaphrodite × staminate); and (3) pistillate (wild type) × staminate (from the cross hermaphrodite × staminate). There were no hermaphrodites among the 131 plants from 12 crosses which blossomed, but there are five plants yet to flower. In all three types of crosses, there was an excess of staminate plants, ranging from three to six times the number of pistillate plants. No hermaphrodites were recovered in another type of cross: pistillate × staminate, both of which originated by self-pollinating hermaphrodites (from H^2). Twenty-three of the 24 plants have flowered.

Although hermaphrodites (from H^2) were involved either directly or indirectly in four types of crosses, no hermaphroditic progeny resulted from pollen of staminate plants. This might indicate that the H^2 hermaphrodite also arose as a mutation of a staminate plant.

Several genetic factors apparently control the flower type in muscadine grapes. Patel and Olmo[7] reported that the chromosome numbers of nearly all Vitaceae are either multiples of six and seven or combinations of them. They identified genomes of 13 and seven in *Vitis rotundifolia*, which would suggest that it is a hexaploid species. Multiple factors as in hexaploids give complicated inheritance ratios.

It has been obvious for many years that it should be possible to originate muscadines essentially homozygous for perfect flowers. However, 36-2A is the first known vine of that type. Larger progenies of 36-2A may produce a few vines which are not hermaphroditic.

The use of parent vines homozygous or nearly homozygous for the perfect-flowered character would increase the percentage of perfect-flowered seedlings in crosses with pistillate or other perfect-flowered varieties. In crosses involving pistlliate vines, entire populations would be perfect-flowered instead of the 50 percent obtained when a non-homozygous perfect-flowered parent is used. The elimination of the pistillate type would result in a tremendous saving of time, labor, and space for it takes two or three years after the vines are planted in the vineyard before they produce flowers.

Literature Cited

1. Dearing, Charles. Muscadine grape breeding. *Jour. Hered.* 8: 401-424. 1917.

2. ————. The production of self-fertile muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 14: 30-34. 1918.

3. Detjen, L. R. Inheritance of sex in *Vitis rotundifolia*. *N. C. Agr. Expt. Sta. Tech. Bul.* 12. 1917.

4. Husmann, George C. and Charles Dearing. The muscadine grapes. *U.S.D.A. Bur. of Pl. Ind. Bul.* 273. 1913.

5. Loomis, N. H. A note on inheritance of flower type in muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 52: 276-278. 1948.

6. ————, C. F. Williams and M. M. Murphy. Inheritance of flower types in muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 64: 279-283. 1954.

7. Patel, G. I. and H. P. Olmo. Cytogenetics of vitis: I. The Hybrid *V. vinifera* × *V. rotundifolia*. *Amer. Jour. Bot.* 42: 141-159. 1955.

8. Reimer, F. C. and L. R. Detjen. Self-sterility of the Scuppernong and other muscadine grapes. *N. C. Agr. Expt. Sta. Tech. Bul.* 209. 1910.

THE INDEX

TO THIS VOLUME HAS BEEN REMOVED

FROM THIS POSITION AND PLACED AT

THE BEGINNING OF THE FILM FOR THE

CONVENIENCE OF READERS.

Mouse. Martinus Nijhoff, The Hague. 1952.
4. Little, C. C. The relation of yellow coat color and black-eyed spotting of mice in inheritance. *Genetics* 2: 433-444. 1917.
5. ————. The inheritance of black-eyed white spotting in mice. *Amer. Nat.* 49: 727-740. 1915.
6. Strong, L. C. The establishment of the C3H inbred strain of mice for the study of spontaneous carcinoma of the mammary gland. *Genetics* 20: 586-591. 1935.
7. ————. The genetic appearance of spontaneous carcinoma of the mammary gland in the C3H mice. *Amer. Jour. Cancer* 25: 599-606. 1935.
8. ————. The establishment of "A" strain of inbred mice. *Jour. Hered.* 27: 21-24. 1936.
9. ————. The origin of some inbred mice. I. *Cancer Res.* 2: 531-539. 1942. II. Old Techniques and New. *Jour. Nat. Can. Inst.* 15: 1417-1425. 1955.

◆

A New Genetic Flower Type of the Muscadine Grape

(*Continued from page 294*)

The one staminate vine is hard to explain. However, in all crosses involving staminate plants, the staminate factor has shown complete dominance over the hermaphroditic factor. Records are available on three different types of crosses involving hermaphrodites from H^2: (1) hermaphrodites × normal (wild-type) staminates; (2) the backcross—hermaphrodites × staminates (from the cross hermaphrodite × staminate); and (3) pistillate (wild type) × staminate (from the cross hermaphrodite × staminate). There were no hermaphrodites among the 131 plants from 12 crosses which blossomed, but there are five plants yet to flower. In all three types of crosses, there was an excess of staminate plants, ranging from three to six times the number of pistillate plants. No hermaphrodites were recovered in another type of cross: pistillate × staminate, both of which originated by self-pollinating hermaphrodites (from H^2). Twenty-three of the 24 plants have flowered.

Although hermaphrodites (from H^2) were involved either directly or indirectly in four types of crosses, no hermaphroditic progeny resulted from pollen of staminate plants. This might indicate that the H^2 hermaphrodite also arose as a mutation of a staminate plant.

Several genetic factors apparently control the flower type in muscadine grapes. Patel and Olmo[7] reported that the chromosome numbers of nearly all Vitaceae are either multiples of six and seven or combinations of them. They identified genomes of 13 and seven in *Vitis rotundifolia*, which would suggest that it is a hexaploid species. Multiple factors as in hexaploids give complicated inheritance ratios.

It has been obvious for many years that it should be possible to originate muscadines essentially homozygous for perfect flowers. However, 36-2A is the first known vine of that type. Larger progenies of 36-2A may produce a few vines which are not hermaphroditic.

The use of parent vines homozygous or nearly homozygous for the perfect-flowered character would increase the percentage of perfect-flowered seedlings in crosses with pistillate or other perfect-flowered varieties. In crosses involving pistlliate vines, entire populations would be perfect-flowered instead of the 50 percent obtained when a non-homozygous perfect-flowered parent is used. The elimination of the pistillate type would result in a tremendous saving of time, labor, and space for it takes two or three years after the vines are planted in the vineyard before they produce flowers.

Literature Cited

1. Dearing, Charles. Muscadine grape breeding. *Jour. Hered.* 8: 401-424. 1917.
2. ————. The production of self-fertile muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 14: 30-34. 1918.
3. Detjen, L. R. Inheritance of sex in *Vitis rotundifolia*. *N. C. Agr. Expt. Sta. Tech. Bul.* 12. 1917.
4. Husmann, George C. and Charles Dearing. The muscadine grapes. *U.S.D.A. Bur. of Pl. Ind. Bul.* 273. 1913.
5. Loomis, N. H. A note on inheritance of flower type in muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 52: 276-278. 1948.
6. ————, C. F. Williams and M. M. Murphy. Inheritance of flower types in muscadine grapes. *Proc. Amer. Soc. Hort. Sci.* 64: 279-283. 1954.
7. Patel, G. I. and H. P. Olmo. Cytogenetics of vitis: I. The Hybrid *V. vinifera* × *V. rotundifolia*. *Amer. Jour. Bot.* 42: 141-159. 1955.
8. Reimer, F. C. and L. R. Detjen. Self-sterility of the Scuppernong and other muscadine grapes. *N. C. Agr. Expt. Sta. Tech. Bul.* 209. 1910.

EUGENICS QUARTERLY

Editorial Board: Frederick Osborn (Chairman), C. Nash Herndon, M.D., Frank Lorimer, Helen G. Hammons (Managing Editor)

VOL. 4, NO. 4 CONTENTS DECEMBER, 1957

American Triracial Isolates: Their Status and Pertinence to Genetic Research, CALVIN BEALE

Blood Groups and Disease: A Promising Field of Medical Research, R. B. MCCONNELL, M.D.

Heredity Counseling: Genetic Clinics and Their Preventive Medical Implications, CHARLES F. WILKINSON, JR., M.D.

Patterns of Discovery in Mental Deficiency, GORDON ALLEN, M.D.

Family Size and Social Mobility in a California Suburb, STEPHEN T. BOGGS

A Reading List on Eugenics, AMERICAN EUGENICS SOCIETY

Eugenic Trends at Mid-century, HELEN HAMMONS

Book Reviews

Periodical Reviews

Genetics, GORDON ALLEN, M.D.

Population, GEORGE F. MAIR

Contributed articles welcomed

Published by

THE AMERICAN EUGENICS SOCIETY, INC.
230 Park Ave., New York 17, N. Y.

Membership in the Society: $5.00 a year including subscription (foreign $2.50)
Subscription to Eugenics Quarterly: $3.00 a year

POPULATION STUDIES

A Journal of Demography

Edited by D. V. GLASS and E. GREBENIK

Vol. XI, No. 2 CONTENTS November 1957

H. C. BROOKFIELD. Mauritius: Demographic Upsurge and Prospect

BERT F. HOSELITZ. Population Pressure, Industrialization and Social Mobility

W. STYS. The Influence of Economic Conditions on the Fertility of Peasant Women

BERTRAM HUTCHINSON. Some Evidence Related to Matrimonial Selection and Immigrant Assimilation in Brazil

J. N. SINHA. Differential Fertility and Family Limitation in an Urban Community of Uttar Pradesh

CHRISTOPHER TIETZE and CLYDE E. MARTIN. Fœtal Dealths, Spontaneous and Induced, in the Urban White Population of the United States

BOOK REVIEWS

Subscription price per volume of 3 parts 35s. net, post free (or American currency $5.75).

Single parts 15s. each plus postage (American $2.50, post free).

Published by the Population Investigation Committee,
at the LONDON SCHOOL OF ECONOMICS AND POLITICAL SCIENCE,
15 HOUGHTON STREET,
LONDON, W.C.2.

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks.

Its membership is composed of men of science, teachers, publicists, physicians, clergymen, parents, students, horticulturists, and breeders of live stock throughout the world.

The Association owns the JOURNAL OF HEREDITY, which is published bi-monthly and sent to each member without additional cost. Every member is thus a part owner of the JOURNAL.

Membership imposes no burdensome obligations. The Association, which is co-operative in nature, welcomes assistance in research, but does not demand it. Members are invited to submit discussions of the results of their research, accompanied by new and unusual photographs. All papers received will be given full consideration by the editorial board.

Manuscripts should be sent to the Editorial Office of the JOURNAL, 1507 M Street, N. W., Washington 5, D. C.

Proof: In order to facilitate prompt publication only galley proof will be sent to authors.

Reprints: On request the author will receive gratis 100 reprints without covers. Additional copies will be furnished at cost.

REQUIREMENTS FOR MEMBERSHIP

Subject to the approval of the Council, any person interested in the improvement of the human race or the creation of better varieties of plants and animals, is eligible for membership.

The Association welcomes all who are interested in its program, and the Secretary will be glad to answer any inquiries.

Annual dues, giving the right to attend all meetings, and receive the JOURNAL OF HEREDITY, are $5.00 within the United States and its possessions; $5.25 in Canada, and $6.00 in all other foreign countries; life membership, $100. Subscription to the JOURNAL is $7.00 per year (foreign postage extra.)

If you are not already a member, and want to become one, or if you know of anyone who would be interested in membership, write to

THE AMERICAN GENETIC ASSOCIATION

Washington 5, D. C.